FINAL REPORT

Project Title

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

Project Supported by
Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)

Executed by
International Center for Tropical Agriculture (CIAT)
in collaboration with
University of Hannover, Germany
Institut des Sciences Agronomiques du Rwanda (ISAR), Rwanda
National Department of the Ministry of Agriculture (DARS), Malawi
Instituto Nicaragüense de Tecnología Agropecuaria (INTA), Nicaragua

Reporting Period
April 2006 – March 2010

CIAT
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INSTITUTIONS AND NETWORKS

BILFA	Bean Improvement for Low Soil Fertility in Africa
BIWADA	Bean Improvement for Water Deficit in Africa
CIAT	Centro Internacional de Agricultura Tropical
DARS	National Department of the Ministry of Agriculture
ECABREN	East and Central African Bean Research Network
INTA	Instituto Nicaragüense de Tecnología Agropecuaria
ISAR	Institut des Sciences Agronomiques du Rwanda
NARS	National Agricultural Research Systems
NGOs	Nongovernmental Organizations
PABRA	The Pan-African Bean Research Alliance, a consortium of national bean research programs, donor agencies, and CIAT, serves to catalyze bean-based work in 18 different countries of East, Central and Southern Africa. NARS, NGOS, farming communities and the private sector are embraced within the alliance.
PROFRIJOL	Proyecto Regional de Fríjol para Centro América, México y el Caribe
SABRN	Southern African Bean Research Network

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Common bean

Brachiaria

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Final Report

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

1. **Name of IARC**
   Centro Internacional de Agricultura Tropical (CIAT)

2. **Project title**
   Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

3. **GTZ Project Number and Contract Number**
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4. **Reporting period**
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   CIAT-Africa
   - Dr. Rowland Chirwa, Bean breeder and coordinator, Southern Africa Bean Research Network (SABRN); Dr. Louise Sperling, Socio-Economist; Dr. Robin Buruchara, Plant Pathologist and PABRA Coordinator; Dr. Paul Kimani, Bean Breeder (ECABREN); Mr. Jean Claude Rubyogo, Bean Agronomist; Dr. Ralph Roothaert, Forage Agronomist (left CIAT in 2007); Dr. Brigitte Maass, Forage Agronomist

   CIAT-Central America
   - Dr. Axel Schmidt, Regional Research Leader (left CIAT in 2009); Dr. Rein Van Der Hoek, BMZ/CIM Expert on Forages

6. **Collaborating institutions and staff including NARS and German partners**
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7. Project description

Goal: To contribute to food security and sustainability of crop-livestock systems in tropical areas prone to drought stress and aluminum (Al) toxicity.

Project purpose: To discover drought and Al resistance candidate genes that are involved in maintaining root elongation under stress; to develop phenotypic and molecular tools to facilitate marker assisted selection (MAS) in common bean and *Brachiaria*; and to increase benefits to resource-poor farmers from stress-adapted, improved common bean and *Brachiaria*.

Project outputs: CIAT has already established strong partnerships with universities, NARS, NGOs and community-based groups; verified robust participatory research and development (R&D) techniques; identified drought and Al-resistant bean and *Brachiaria* germplasm; created breeding populations; and initiated the development of the mapping populations needed for the success of the project. The project will deliver the following intermediate outputs over a three-year period: (1) Rural benefits enhanced in the target areas of tropical Africa and Central America by involving farmers as decision makers and co-researchers in the common bean and *Brachiaria* product development process; (2) Physiological mechanisms characterized in contrasting parents, interactions defined, and screening methods developed, for: root traits, water and nutrient uptake and transport, and shoot effects under individual or combined stress factors of drought and Al toxicity; (3) Genomic tools (gene libraries and cDNA microarrays) deployed to analyze the effects of drought and Al toxicity on expression of genes involved in root elongation, and to identify candidate genes responsible for drought and Al resistance in common bean and *Brachiaria*; (4) Improved genetic adaptation of common bean and *Brachiaria* to drought and Al toxicity, through deployment of phenotypic screening methods to develop DNA markers; and (5) Capacity of students, NARS researchers, and farming communities enhanced in stress physiology, functional genomics, molecular plant breeding and participatory R&D methods.

8. Major Research Findings

Output 1: *Rural benefits enhanced in the target areas of tropical Africa and Central America by involving women and small-scale farmers as decision makers and co-researchers in the common bean and Brachiaria product development process*

ISAR-Rwanda: Beans

Participatory selection of drought resistant bean varieties: Two drought tolerant bush bean varieties, SER 16 and SER 30 were released to farmers. They are shiny red coated and averaged a yield potential of 2.0 – 2.5 t/ha. Besides high yield and drought resistant, the varieties were appreciated by farmers for the early maturity, good taste, fast cooking and the fact that they do not stain cookers. They made red-brown source similar to that of meat's that had good taste and flavor, especially in popular mixed dishes with tubers and cereals, locally called (Agatogo). They were baptized as Munyu 1 (SER 16) and Munyu 2 (SER 30). Munyu means salty or good taste.

At the same time, 3 heat and drought tolerant climbing bean varieties adapted to low altitude and warmer zones of eastern Rwanda: MAC 9, MAC 44, and MAC 49 were also released. They had mean yield potential of 3.3 to about 4.0 t ha^{-1}. This was about 2 to 2.5 times more than the yield of the popularly grown mixed bush variety (local mixture) that was used as a check. They were relatively early maturing (85 – 87 days), compared to climbers adapted to higher altitude 1600 –

2300 masl, that take more than 100 – 120 days to mature. Besides being resistant/tolerant to major diseases, they were appreciated by farmers due to their medium or large red mottled (Calima) seed types. Farmers associate them with fast cooking, appetizing and tasty broth as well as premiums on the market. They were released (in January 2010) under farmers' names: Gitego 1 (MAC 9), Gitego 2 (MAC 44) and Gitego 3 (MAC 49). "Gitego" is a local word, meaning "score".

Participatory evaluation and selection among 60 drought resistant and advanced drought resistant lines over seasons and years, resulted in selection of 7 pre-release drought resistant bush and semi-climbing bean varieties for the semi-arid zones of eastern Rwanda (Bugesera and Umutara): SER 12, SER 13, SER 14, NR1467-17-6P, MR14258-7-8P, MR14265-58-2P and TIO CANELA 75. They were selected for superior yield and early maturity compared to the released varieties and local checks.

Photo 1. One of the drought resistant varieties, SER 16 that was released by ISAR in Rwanda in January, 2010: in the field (left) and its grain (right).

Participatory selection of acid soil adapted lines: Among the advanced Al-toxic acid soil and/or drought adapted bean lines, the following lines that showed greater potential for adaption to acid soil stress conditions in Gikongoro/Nyamagabe in southern Rwanda were selected: CIM-RM-OO-323-O2, CIM-RM-OO-321-O3, VTTT924/2/2-4-2-1, SEN 13, SEN 23, SEN 24, ENTRY 18, and ENTRY 20. They will be further tested by ISAR-Rwanda in more locations for further evaluation for resistance and adaptability and the most elite ones released to farmers and/or used in crossing program to improve the resistance of the commercial varieties.

Farmers' preferences and selection criteria of drought resistant varieties: ISAR bean program scientists and technicians (8) and 23 other representatives of the project partners from NGOs, Farmers' Cooperatives and Ministry of Agriculture and Extension Services were trained and acquired skills to conduct successful Participatory Varietal Selection (PVS). They all participated in the PVS with farmers in the different on-station and on-farm/multilocation trials over seasons. During the various PVS sessions, diverse criteria in selection were recorded, including specific drought resistance-related insight. The most frequently mentioned criteria were: early uniform maturity (to escape terminal drought); green leaf canopy where others are wilting/dried (as a sign of drought resistance); strong and firm stems that don't log easily (associated with deep root system); strong and firm stems that can support heavy pod loads and pods that are uniformly distributed from the base upwards (upper pods preferred under rainy conditions).

Other general (newly heard) supplementary selection criteria that were mentioned were: green pods that are easy to open/unshell (some fresh pods take time and energy to open); bean seed types that can be cooked with "one log" of wood (very fast cooking observed by certain seed-types); small and heavy seeds associated with high density (mass per unit volume) that is good for higher profits once sold by weighing balance, but at loss once sold by sacs (volume); and, conversely, large seeded that fetch higher prices by sacs and less by weighing; some bean types

are apparently “oily” and won’t stick to local cookers after cooking and so easy to clean (as opposed to some, such as black beans that coat cookers with hard stains that are hard to remove). Others were: clear/shiny red seed coat associated with “blood” (meaning good taste and nutritional quality), and those that are light colored and cause low or no flatulence (yellows, khakis, white or light colored)

Overall, the project succeeded in the selection and release of the new drought resistant SER (small red) lines, SER 16 and SER 30, and contributed to the release of the first varieties of climbing beans (MAC lines- MAC9, 44 and 49) that are suitable for the lower elevation zones of the target area of the project. This is within limits, or even beyond, the project planned outputs. One key factor was the adequate selection pressure (for drought stress) and farmers’ early involvement in the exercise that speeded up identification of truly adapted and accepted criteria as well as the varietal release process itself. Leveraged funding and support of The Alliance for a Green Revolution in Africa (AGRA) and PABRA, and Pulses CRSP breeding projects contributed the speedy, selection and release of the bush and climbing bean varieties.

Much was also achieved under PVS, in terms of acquiring new skills and documentation of the new criteria that farmers use during selection for drought resistant bean lines. The protocol used, especially for disaggregating gender (men and women choices) in selection by using different colored ribbons; secret placement of ribbons into opaque bags, open discussions after results of preferences, was added value to the previously used open variety selection protocol. The latter protocol made PVS exercise more purposeful and effective.

However, the project fell short of identifying acid soil adapted bean lines through PVS in Rwanda. Thus selection for acid soil adaptation needs to be continued. At the institutional level, the project was constrained by staff and leadership changes due to training (Louis Butare and Alice Kabana), and departures (Felicite Nsanzabera and Dolphin Kambayire). However, a major effort was made to execute project activities.

DARS-Malawi: Beans

Participatory selection of drought resistant bean varieties: DARS also has a strong history in PVS training and techniques. Four Malawian scientists were among the 25 professionals trained in a 10-country (Malawi, Uganda, Mozambique, Zimbabwe, Zambia, Kenya, Burundi, DRCongo, Tanzania and South Africa) Training of Trainers (ToT) course in September 2008. Forty common bean lines were evaluated for drought resistance at Kasinthula Research Station (under both irrigated and stressed conditions) and 26 bean lines were identified to be further evaluated in a multilocational trial. Using PVS, 100 common bean lines were evaluated on-farm in Kasungu (at Chinseu and Bokosi villages) for drought resistance, out of which men and women farmers selected 17 lines they considered to have the following traits: high grain yield, large grains, bright and attractive colors for the market. Women additionally considered criteria such as early maturity, cooking time and taste.

Participatory selection of acid soil adapted lines: Using PVS at Bembeke, 40 bean lines were evaluated for Al resistance from which 5 superior lines were identified with SX14331 and SEN 39 being the most liked by the farmers, both men and women. The results have shown that farmers prefer VTTT925/9-1-2, BF 13572-5 and SER 83 and these three lines also yielded higher than the control CAL 143 under drought stress. Under Al toxicity the following three lines gave high yields showing that they were Al resistant: SER 55, SER 83 and SER 75.

Farmers’ preferences: Two bean genotypes, VTTT925/9-1-2 and BF 13572-5, have been selected by farmers based on good table and market traits and these two genotypes also have high yielding potential under stress conditions. Hence both have been recommended for release so that the farmers can start multiplying and diffusing them widely.

ISAR-Rwanda: Forages

Participatory evaluation of Brachiaria grass options in Rwanda: Both the number and type of cattle are important in defining wealth ranking and status within the community in contrasting drought (Bugesera district) and acidic soil (Nyamagabe district) environments of Rwanda.

Livestock activities are usually shared between genders, but certain activities (e.g., milking cows, animal shed construction) are restricted to males due to cultural beliefs, while rearing of small livestock is only carried out by women and children.

Napier grass is currently the major feed source used throughout the two Rwandan districts. Farmers' perceptions of feed availability, quality and quantity showed a shortage of livestock feed resources especially during the dry season or unforeseen drought periods.

Herbage dry matter (DM) production of different *Brachiaria* cultivars and hybrids was assessed in on-farm participatory trials by 12 farmers in each region. In the drought-prone district, *B. brizantha* cv. Toledo and local *B. decumbens* produced the highest mean DM yields (> 5.6 t ha^{-1} per 2-month interval), which was particularly due to their good dry-season performance. In the acidic soil area, *Brachiaria* hybrid BR02/1485 had superior mean DM yields of almost 6 t ha^{-1} per 2-month interval, together with *Brachiaria* hybrid cv. Mulato II showing superior dry-season performance. The same hybrid (BR02/1485) showed the highest crude protein (CP) content, while cv. Mulato II was outstanding in the acidic soil area (12.2% and 11.6% CP, respectively). This level of nutritional quality is clearly superior to that of the local grasses that are presently in use.

Farmers' selection criteria were similar in the two Rwandan districts targeted. However, in the drought-prone district the criterion of persistence was stressed, whereas in the acidic soil area disease resistance was emphasized. In the former district, because of feed scarcity during the dry season, cv. Mulato II was preferred as a forage resistant to drought, followed by *B. brizantha* cv. Marandu, *B*. hybrid cv. Mulato and *B. decumbens* cv. Basilisk. In the latter district, farmers ranked *B.* hybrid BR02/1485 (Photo 3), cv. Mulato II (Photo 2), and local *B. decumbens* were identified as superior performers, followed by cvs. Basilisk and Toledo (Photo 4). Despite cv. Mulato II not being the most productive grass, it was selected by farmers at both sites because of its adaptability to low rainfall and acidic soil stress, as well as its production of green forage year round without any fertilizer input.

Photo 2. *Brachiaria* hybrid (cv. Mulato II)

Photo 3. *Brachiaria* hybrid (BR02/1485)

Photo 4. *Brachiaria brizantha* (cv. Toledo)

Based on a survey in both districts, 40-65% of farmers directly involved in the project have adopted new forages by increasing the size of their plots and forming cooperatives to produce planting materials of the best bet *Brachiaria* grass options. Several additional farmers have acquired these new grasses.

INTA-Nicaragua: Forages

Participatory evaluation of Brachiaria grass options in Nicaragua: A field trial with five hybrids (three new and two recently introduced commercial) and three cultivars of *Brachiaria* spp. was carried out in the municipality of Diriamba, located in an extensive dry zone in Nicaragua's Pacific region, characterized by a large shortage of forages during a 5 months dry season. During the dry season *Brachiaria* hybrid CIAT 36087 (cv. Mulato II) showed the highest forage yield (4.1 t ha^{-1} of dry matter at a cutting interval of five weeks, $p<0.05$) while the new *B*.

hybrid BR02/1452 and cultivar *B. decumbens* CIAT 606 showed lowest forage yields (both 1.5 t ha^{-1} of dry matter). During the wet season, no differences ($p>0.05$) in average dry matter production were found among all hybrids and cultivars; however, *Brachiaria* hybrid CIAT 36061 (cv. Mulato) tended to show superior wet season performance followed by B*. brizantha* CIAT 26110 with 6.0 and 5.2 t ha^{-1}, respectively. Farmers mostly appreciated *Brachiaria* hybrid CIAT 36087 (cv. Mulato II) because of its high forage yield, soft stem and leaves and its capacity to recover from severe *Rhizoctonia* and spittlebug infestation.
Detailed report on activities of Output 1 is provided as Annex-1.

Output 2: *Physiological mechanisms characterized in contrasting parents, interactions defined, and screening methods developed, for: root elongation, water and nutrient uptake and transport in roots and effects on shoot growth under individual or combined stress factors of drought stress and Al toxicity*

Physiological and molecular characterisation of aluminium resistance: The physiological characterization of two common bean (*Phaseolus vulgaris* L.) genotypes differing in aluminium (Al) resistance, Quimbaya (Al-resistant) and VAX-1 (Al-sensitive) had revealed that root elongation of both genotypes was severely inhibited during the first 4 h of Al treatment. Thereafter, both genotypes showed gradual recovery. However, this recovery continued in genotype Quimbaya until the root-elongation rate reached the level of the control (without Al) while the genotype VAX-1 was progressively damaged by Al after 12 h of Al treatment. Aluminium treatment enhanced the exudation of citrate from the root tips of both genotypes. However, its dynamic offers the most consistent relationship between Al-induced inhibition of root elongation and Al accumulation in and exclusion from the root apices. Sustained recovery from Al stress through citrate exudation in the Al-resistant common bean genotype Quimbaya after 24 h Al treatment relied on restoring the internal citrate pool and the constitutively high activity of citrate synthase fuelled by high phosphoenolpyruvate carboxylase (PEPC) activity.
After the clarification of the physiology of Al toxicity and Al resistance in beans, we concentrated on the molecular studies of these processes in the Al-sensitive genotype VAX-1 and the Al-resistant genotype Quimbaya. At first, we used a proteomic approach on root tips the main site of Al injury. Much to our surprise, 2D IFS/SDS page did not reveal any differences in protein patterns in spite of major physiological changes as revealed by severe inhibition of root elongation after 4h of Al supply and beginning of recovery of root elongation after 12h Al supply. Also high resolution SDS page which better separates hydrophobic membrane proteins indicated changes in the formation of individual proteins only after 24h Al supply in the Al-sensitive genotype. Present studies continue focussing of the characterization on protein activity through phosphor-proteomics and the proteomic characterisation of proteins in mitochondria the organic acid synthesising cell compartment.
In a first step, the Suppression Subtractive Hybridization (SSH) method was used to identify differentially expressed genes in the Al-resistant bean genotype Quimbaya during the induction period. In a second step the expression patterns of identified and candidate genes were compared between the Al-resistant and the Al-sensitive genotypes Quimbaya and VAX 1 treated with Al for up to 24 hours using quantitative real-time PCR (qRT-PCR). We identified genes encoding citrate transporters in beans. The ESTs belonging to the Multidrug And Toxin Extrusion (MATE) family protein were found to be highly expressed after about four hours of Al treatment. Citrate exudation and the resulting Al resistance phenotype can only be observed after the expression of these genes indicating that these genes are crucial for citrate transport across plasma membrane. These results have been published in an international Journal.
Relationship between aluminium toxicity and drought stress: Polyethylene glycol (PEG-6000) a high molecular weight polymer was used to modify the osmotic potential of nutrient solution and thus induce plant-water deficit under controlled conditions. Genotypes differed in the response to PEG stress. However, the most significant finding from studies on the interaction of

Al toxicity and PEG-induced drought stress was that PEG treatment improved Al tolerance in Al-sensitive but not in Al-resistant genotypes, which was accompanied by the exclusion of Al from uptake into root apical tissues. An in-depth study of this phenomenon in Al-sensitive genotype VAX-1 revealed that PEG 6000-induced osmotic (drought) stress inhibited Al accumulation in root apices and thus reduced Al-induced inhibition of root elongation is related to the alteration of cell wall (CW) porosity resulting from PEG 6000-induced dehydration of the root apoplast. These results have been published in an international Journal.

Phenotyping protocols for evaluating Al resistance: Phenotyping protocols for evaluating Al resistance were improved by developing filter paper/Styrofoam sandwich system for seed germination, by modifying the marking system for measuring rate of root elongation and by adapting the agarose-based method to detect Al-chelating compounds exuded from roots.

Al resistant bean genotypes: Screened 19 genotypes for Al resistance in nutrient solution and found that the level of Al resistance in ICA Quimbaya was superior to the tested intraspecific (Andean x Meso) and interspecific (*P. vulgaris* x *P. acutifolius*) lines. Screened 15 bean genotypes for aluminium resistance and identified 5 runner bean (*Phaseolus coccineus*) genotypes (G 25448-7P G 35066-1Q, G 35777-2P, G35448-10P, G 35777-3P) with high level of resistance to Al in solution and 4 runner bean genotypes (G 35448-10P, G 35448-5P, G 35448-1P and G 35777-6P) with greater values of total root length production under both with and without Al in solution. Screened 72 RILs of ICA Quimbaya x VAX 1 and identified several RILs that were superior to Al resistant parent Quimbaya.

Deep rooting bean genotypes: Greenhouse evaluation of phenotypic differences among 16 bean genotypes under intermittent and terminal drought stress using soil tube methodology indicated that G 21212 and BAT 4777 were superior under terminal drought stress while BAT 881 was superior in intermittent drought stress based on total root length in 80 cm long soil tubes. *P. acutifolius* (G 40159) showed fine and deep root development under drought stress while the accessions of *P. coccineus* (G 35066 and G 35884) showed thicker and less deep rooting under drought stress. Greenhouse evaluation of phenotypic differences among 13 bean genotypes under high Al stress in soil tubes indicated that *P. coccineus* G 35448-9P was outstanding in total root length and deep root development in Al toxic soil.

Shoot traits related to drought resistance: Field evaluation of 36 elite bean lines for their level of resistance to intermittent drought stress identified 2 black seeded lines (SEN 56, NCB 226) that were outstanding in seed yield under stress and were also responsive to irrigation. The superior performance of these two lines was associated with very high values of pod harvest index.

Bean genotypes resistant to combined stress of drought and Al-toxic acid soil: Greenhouse evaluation to determine the influence of combined stress factors of high Al and drought in an acid soil on root development and distribution of eleven genotypes of different *Phaseolus* species grown in soil tubes resulted in identification of one genotype of *Phaseolus coccineus* (G 35884 1Q) that was superior in root development under high Al stress and also the combined stress of high Al and drought.

Al resistant Brachiaria hybrids: Screened 79 most promising *Brachiaria* hybrids (developed by the breeding program over the past 6 years) for their resistance to Al in nutrient solution and identified 7 hybrids (BR04/02069, BR05/00334, BR05/00563, BR06/0012, BR06/0531, BR06/1175, BR06/1278) with high root vigor and higher level of Al resistance. These hybrids also combine some level of resistance to major insect pests called spittlebugs and therefore could be candidates for further agronomic evaluation as potential cultivars to release.

Acid soil adapted Brachiaria hybrids: Phenotypic differences among 12 *Brachiaria* genotypes (*Brachiaria* hybrid Mulato, *Brachiaria* hybrid Mulato II, *B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02, BR02/1752, SX03/2367, SX03/846, BR02/1485, BR02/465, Br02/1372, SX03/881) in root development under Al toxic soil conditions were evaluated using

soil tubes and it was found that the *B. decumbens* CIAT 606 and the *Brachiaria* hybrid BR02/1372 were superior in total root length under Al stress.

Drought resistant Brachiaria hybrids: Phenotypic differences among 12 *Brachiaria* genotypes (*Brachiaria* hybrid Mulato, *Brachiaria* hybrid Mulato II, *B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02, BR02/1752, SX03/2367, SX03/846, BR02/1485, BR02/465, Br02/1372, SX03/881) in root development under intermittent and terminal drought stress were evaluated using soil tubes and it was found that the *Brachiaria* hybrid Mulato was superior in total root length under both terminal and intermittent drought stress conditions. Rooting ability in deeper soil layers showed significant correlation with drought resistance.

Detailed report on activities of Output 2 is provided as Annex-2.

Output 3: *Genomic tools (gene libraries and cDNA microarrays) deployed to analyze the effects of drought and Al toxicity on expression of genes involved in root elongation, and to identify candidate genes responsible for drought and Al resistance in common bean, runner bean, and Brachiaria*

Aluminum resistance genes in common bean: Phenotypic evaluation of the Al-resistant and Al-sensitive common bean genotypes indicated huge differences in root development suggesting a strong Al tolerance for the former, mainly at 48 h of Al exposure. A total of 17 differentially expressed bean genes were isolated from the subtracted cDNA library. Likewise, several mechanisms likely induced by Al toxicity were also predicted through BLAST analysis from the sequences of these genes, namely diphosphate kinase, glucanase, among others.

Release of root organic acids (Al-activated malate transporter and citrate transporter) has been suggested as a mechanism of Al tolerance. Primers based on key genes for these mechanisms were designed. To isolate *ALMT1* genes in common beans, degenerated primers were designed based on the conserved amino acid sequences between ALMT1 in *T. aestivum* and the predicted homologs in *A.thaliana* (At3g11680 and At1g08440) and *O. sativ*a (Os04g47930 and Os02g45160).

According to phylogenetic analysis, we identified 4 homologues of *HvAACT1* gene and 2 homologues of *STOP1* gene from the *P. vulgaris* EST database. Early Al stress (4h) induced the expression of those homologous genes in both resistant and sensitive bean genotypes. But, while with Al treatment duration, it decreased the expression level in resistant genotype while it continued to keep the higher expression level in sensitive genotype.

Through data mining of updated literature and *in silico* analysis (bioinformatics) of the gathered information, a standard operating procedure was embraced in order to look for master genes related to abiotic stress response and root development. The expected outcome of this scheme is to link the sequence information of heterologous species with beans and soybean and thereby design primers over key homolog genes for future characterizations.

By using bioinformatics tools, a set of primers for master genes known to be involved in Al-resistance in roots was generated for gene expression analysis in beans, i.e., STOP 1 (Sensitive to proton rhizotoxicity), ATR (ataxia telangiectasia-mutated and Rad3-related), MDH (malate dehydrogenase), ACS (1-aminocyclopropane-1-carboxylic acid [ACC] synthase) and ACO (1-aminocyclopropane-1-carboxylic acid [ACC] oxidase), ATP binding cassette (ABC) transporter, CS (citrate synthase), ALMT1 (Aluminium Activated Malate Transporter), MATE (Multidrug And Toxic compound Extrusion), and Δ-8 SLD1 (sphingolipid desaturase).

Drought and aluminum resistance genes in common bean: We identified some candidate genes to be involved in drought and Al resistance in beans by constructing subtractive cDNA libraries. A drought resistant genotype, G 40159 (*Phaseolus acutifolius*), and a drought sensitive genotype, DOR 364 (*Phaseolus vulgaris*), were selected based on their contrasting ability to develop a deep root system under drought conditions. For the Al-experiment, we focused on two bean genotypes based on their contrasting response to Al toxicity. G 35346-3Q (*Phaseolus coccineus*), an Al-resistant genotype, and SER 16 (*Phaseolus vulgaris*), an Al sensitive

genotype, were selected based on their root attributes (root length, number of root tips and vigor) in response to Al-toxicity under hydroponics conditions. As a result of the subtractive screening, we have isolated one and three up-regulated genes in drought and Al resistant genotypes, respectively. The position of the candidate genes for Al-resistance on *P.vulgaris* chromosome was predicted using the synteny between soybean and common bean. Some of the candidate genes were matched to the postion of QTL for Al-resistance. Transcriptional changes in bean root tips due to Al stress were studied to find out genes which are responsible for Al resistance in common bean.

A scheme was established for data mining and *in silico* analysis in order to obtain gene information about legumes and gene networks that are directly related to root development and abiotic stresses with a main focus on regulatory components (i.e., transcription factors).

Aluminum resistance genes in *Brachiaria*: In general, expression of 7 *Brachiaria* candidate genes was similar in the three most tolerant species (*B. decumbens, B. brizantha* cv. Marandu, and *Brachiaria* Hybrid Mulato 2). However, the expression of genes in leaves is not consistent with the expression in root tips. In some cases, there was no differential expression between *B. decumbens* and *B. ruziziensis* regarding leaves and roots subjected to Al exposure nor in root tips of seedlings under NaCl stress.

Several contigs obtained by RACE (Rapid amplification of cDNA ends) revealed the complete sequence of the AlBdec10 *Brachiaria* gene, corresponding to the metal-dependent protein hydrolase family. A probable function for the HD domain of this family corresponds to signal transduction. This family of proteins contains a large number of metal binding residues such as phosphohydrolases, which usually are metalloenzymes that contain metal-chelating residues, typically histidines and aspartates.

A wheat (*T. aestivum*) *ALMT*1 (*Aluminium Activated Malate Transporter*) homolog gene was identified in *Brachiaria.* The differential of expression of this homolog gene between the resistant and sensitive genotype, mainly at the first hours of Al-stress, was not substantial; so this implies this gene is not exclusively involved in Al-resistance in *B. decumbens.* Nevertheless, this gene might be contributing to resistance in *B. decumbens*. This was evident at 48 and 72 hours of Al exposure. On the other hand, *STOP 1* (Sensitive to proton rhizotoxicity), a transcription factor that regulates the expression of *ALMT*1 in *A. thaliana* and is also required for *AtMATE* expression and Al-activated citrate exudation, is not likely to be involved in Al resistance in *Brachiaria* as the expression of the *Brachiaria*-homologous *STOP1* in the sensitive genotype is higher than that the resistant mainly at 24 and 48 hours of Al-exposure. Furthermore, a homolog of *Hordeum vulgare HvAACT1 (Aluminum-Activated Citrate Transporter)* gene was also identified in *B. decumbens.* This gene belongs to the *MATE* (*Multidrug and Toxic Compound Extrusion*) family and is responsible for Al-activated citrate secretion in Al-resistant barley cultivars. We found a close (90%) homology between the sequence of this gene identified in *B. decumbens* and the one of *O. sativa*. Gene expression analysis of this gene in *Brachiaria* is in progress.

Gene expression analysis of 7 differentially expressed *Brachiaria* genes was performed in four *Brachiaria* genotypes at different times of Al exposure (0, 3, 6, 24, 48, and 72 h). This analysis was also carried out in leaves (seedlings grown in solution with Al) and root tips (seedlings grown in solution with NaCl) to identify responses that are specific to Al toxicity. Adopted rapid amplification of cDNA ends (RACE) to obtain a full-length gene from the previously identified 7 candidate genes in *Brachiaria*. Using gene expression profiles, heterologous Al-resistance genes in *Brachiaria* were identified. Homolog sequences of *TaALMT*1*, STOP*1*,* and *HvAACT*1 genes were found in *Brachiaria.* Subsequently, a gene expression analysis was carried out so as to determine the contribution of these genes to Al-resistance in *Brachiaria*.

Detailed report on activities of Output 3 is provided as Annex-3.

Output 4: *Genetic adaptation improved of common bean and Brachiaria to drought and Al toxicity, through deployment of phenotypic screening methods to develop DNA markers*

New sources of resistance in *Phaseolus* species to individual and combined stress factors of aluminum-toxic acid soil and drought: Improved screening methods and sources of resistance would speed progress in breeding for abiotic stress resistance in common bean. Bean species and genotypes showed wide phenotypic variability in resistance to Al and drought stress. We identifed sources of resistance among six genotypes of *Phaseolus vulgaris*, four of *P. coccineus*, and one of *P. acutifolius*, to Al and drought stress using hydroponic and soil tube screening methods. A hydroponic screening of Al resistance was carried out using a basal nutrient solution with and without 20 µM Al. Two experiments in 80 cm long soil tubes were carried out using an oxisol with 76% (pH = 4.1) and 83% (pH = 4.14) Al saturation, for topsoil and subsoil, respectively. The three experiments showed an average of 36.9 to 53.5% inhibition of root growth between high and low Al treatments. Differences in root development and distribution were observed. Two accessions of *P. coccineus* (G35346-2Q, G35464-5Q) and one Andean genotype (ICA Quimbaya) were outstanding in root and shoot growth in the high Al treatments. *P. coccineus* accession (G35346-3Q) was outstanding under combined stress of drought and acid soil. The methodology used to evaluate genotypes for individual and combined stress factors served to identify superior parental genotypes for use in breeding programs.

Phenotypic evaluation of interspecific recombinant inbred lines (RILs) of *Phaseolus* species for their resistance to aluminum and tolerance to aluminum-toxic acid soil under greenhouse conditions: To improve the resistance of common bean to Al, progenies of Al sensitive *Phaseolus vulgaris* (SER16) and an Al resistant *Phaseolus coccineus* (G35346-3Q) including 94 $F_{5:6}$ RILs of the cross SER16 x (SER16 x G35346-3Q) were characterized for their resistance to Al and tolerance to Al-toxic acid soil under greenhouse conditions using hydroponic and soil tube systems. Two levels of Al (0 and 20 µM), were employed for phenotypic evaluation in the hydroponic system while an oxisol from Santander of Quilichao (Colombia) with low Al (12.5%; pH 4.6) and high Al-saturation (77 %; pH 4.1) was used for evaluation with soil tube (75 cm soil depth) system. For the low Al saturation as a control treatment, soil was adequately fertilized. Seedlings of all 102 genotypes (94 RILs, 2 parents and 6 checks) except the donor parent (G35346-3Q) showed reduced root elongation rates under the 20 µM Al treatment in the hydroponic system. Sensitive genotype VAX1 exhibited greatly reduced root growth rate by 69.5% whereby the most Al-resistant lines were G35346-3Q, and RILs ALB32, ALB41, ALB45 and ALB23 with primary root elongation rates reduced by -15.7%, 2.6%, 3.5%, 5.4%, and 8.1%, respectively. The root growth rate of an Andean genotype ICA Quimbaya was reduced by 12.5%. In the soil tube system, more than 50% of the RILs were deeper rooted than the Al sensitive parent SER16. Correlation between leaf area and total root length was highly significant under both high ($r = 0.70$, $P < 0.001$) and low ($r = 0.56$, $P < 0.001$) Al saturation. Genotypic ranking for Al resistance was different between the hydroponic and soil tube systems indicating that the genotypes that are Al resistant are not necessarily tolerant to acid soil conditions. Phenotypic evaluation using both systems allows identifying genotypes with Al resistance combined with acid soil adaptation for both shoot and root vigor. The results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

Evaluation of recombinant inbred line (RIL) populations for drought resistance: Field evaluation of 33 RILs of the cross DOR 364 x BAT 477 at Palmira over two season under terminal drought stress conditions resulted in identification of two lines (BT 21138-17-1-1 and BT 21138-6-1-1) that were superior in their adaptation to drought stress conditions. The superior performance of these lines under drought stress was associated with higher values of harvest index, pod harvest index and seed and pod number per area indicating the importance of greater mobilization of photosyntates to pods and to seeds under rainfed conditions.

Field evaluation of 97 RILs of the cross DOR 364 x BAT 477 under intermittent drought stress resulted in identification of one RIL BT 21138-68-1-1 and BT 21138-74-1-1 that was outstanding in adaptation to intermittent drought stress conditions. The superior performance of this line under intermittent drought stress was associated with higher values harvest index, pod partitioning index, pod harvest index, seed number per area and pod number per area indicating the importance of greater mobilization of photosyntates to pods and seeds under rainfed conditions.

Identification of drought resistant lines: Field evaluation of elite lines at CIAT-Palmira over two seasons under intermittent drought stress resulted in identification of six small seeded common bean lines (NCB 226, SEN 56, SER 113, SER 125, SXB 415 and SER 16) that were outstanding in their adaptation to drought stress conditions. The superior performance of these lines under drought stress was associated with higher values of harvest index, pod harvest index, leaf area index and canopy biomass. The promising small seeded black and red lines that were developed in the last few years seem to combine the desirable traits, for adaptation to intermittent drought stress, such as greater mobilization of photosynthates to seed with efficient use of water through stomatal control.

Identification of acid soil and low phosphorus soil adapted lines: Field evaluation of thirty six bean genotypes including elite lines for their adaptation to acid soil and low phosphorus soil resulted in identification of two Mulatinho lines (SXB 412, SXB 405), two carioca lines (SXB 409, SXB 415), and two small seeded red lines (SER 113, SER 118) that were found to be superior in grain yield. Their superior performance was associated with higher values of pod harvest index and lower seed P content under both acid soil and low P soil conditions.

Development of bean lines combining drought and Al resistance: The Al resistance of *Phaseolus coccineus* accession G35346-3Q was confirmed in both soil and solution culture. The root system of this accession was also resistant to combined stress of drought and acid soil complex. G35346-3Q actually expressed greater root development in soil tubes under combined stress than under drought alone. In solution culture other genotypes gradually slowed tap root elongation over 48 h of 20 µM treatment, but G35346-3Q recovered and accelerated its elongation in the same period. Lines combining drought and aluminum resistance were obtained from a backcross of G35346-3Q to SER 16. Some appear to have improved yield potential under favorable conditions, perhaps reflecting improved root traits that have been introgressed from *P. coccineus* into the common bean parent. Wide variability in root phenotypes occurred among sister lines of the interspecific crosses. This variability reflects introgression from *P. coccineus* to SER 16. *Phaseolus coccineus* accession G35346-3Q is also resistant to at least one serious soil pathogen. Although several crosses were made between common bean lines of the Andean gene pool and *P. coccineus*, these were not as successful as those with SER 16. Several such crosses displayed severe incompatibility in the F1 generation. Although some intraspecific lines developed for resistance to Al toxicity performed well in an acid soil site, these lines did not display as wide adaptability across environments as the interspecific lines.

Among the 90 advanced lines evaluated under intermittent drought, two lines (ALB's 205 and 167) presented yields that were significantly better than that of recurrent parent SER 16 ($p=0.05$), with a slightly longer growth cycle (68 versus 63 days to physiological maturity). ALB 205 was one of a series of F2-derived sister lines that tended to yield well. Of these sisters, ALB 213 presents excellent agronomic habit. These lines appear to have maintained the remobilization capacity and the efficiency in yield per day of SER 16, while lengthening the growth cycle for better yield. The superiority of the lines over elite selections of previous years was notable, especially in relation to BAT 477 which was the elite drought tolerant line two decades ago and which yielded 1000 kg ha^{-1} less that the top lines. Thus, many of the selected lines appear to have recovered the drought tolerance of SER 16. Under aluminum toxicity several were superior to VAX 1 but none significantly out-yielded SER 16, the recurrent parent.

Yield of elite lines derived from intraspecific and interspecific crosses in response to drought and acid soil complex: Yield trials of RILs were carried out under three conditions: acid soil complex, drought, and unstressed irrigated. Substantial yield gain was made in tolerance to Al toxicity over the VAX 1 check, and in multiple stress resistance. Unexpectedly, the interspecific crosses produced progenies with excellent agronomic traits, and unusually good yield potential. We believe that this reflects the enhanced photosynthate remobilization of SER 16 in combination with biomass accumulation and root traits derived from *Phaseolus coccineus*.

Release of 2 drought resistant bean lines: SER 16 and SER 30, both drought resistant common bean lines of the Mesoamerican gene pool, were released in Rwanda.

Phenotypic evaluation of Brachiaria hybrid populations for drought resistance: Screened 221 *Brachiaria* genotypes from BR05 and RZ05 genetic recombinant populations and 41 *Brachiaria* genotypes from SX05 population for their resistance to drought in pots with soil by withholding watering and identified 2 tolerant hybrids from BR05 population (BR05/0048, BR05/0406) and 1 sexual hybrid (SX05/2480) from SX05 population.

Identification of *Brachiaria* hybrids with adaptation to drought: A total of 79 *Brachiaria* genotypes including 71 promising *Brachiaria* hybrids were evaluated for their resistance to drought when grown in pots with high fertilizer application. One *Brachiaria* hybrid, BR06/1932, was found to be outstanding in its drought adaptation as determined by green leaf biomass production and green leaf biomass proportion. But its water use for growth was also greater than that of the other hybrids. Another hybrid, BR06/1922, was identified to be capable of producing greater green leaf biomass proportion with moderate use of soil water.

Identification of *Brachiaria* hybrids with combined stress adaptation to low fertility acid soil and drought: Eleven *Brachiaria* genotypes including parents and promising hybrids were evaluated for root development under individual and combined stress factors of low soil fertility and drought. Two apomictic hybrids (BR02/1752 and BR02/1372) and one sexual hybrid (SX03/2367) were found to be outstanding in their total root length production across soil depth under combined stress factors of low soil fertility and drought.

Identification of QTLs for aluminum resistance in Brachiaria: A genetic linkage map was constructed for *Brachiaria decumbens* using 122 molecular markers including SSR, EST, AFLP and SCARs., on a hybrid population (generated from a cross of two *Brachiaria* parents) with eighteen linkage groups that covered 1094 cM (mean 60.7 cM per linkage group). Five putative QTLs were found in five different linkage groups (LG1, LG3, LG4, LG7 and LG17); these QTLs explained the 6.6%, 7.8%, 11%, 13% and 21% of the aluminum stress response respectively. It is necessary to continue with the saturation of the linkage map to increase the power of detection of genome regions that govern the quantitative characteristics of aluminum stress. Additional investigations into the physiological mechanisms, pathway interactions, systems biology and detailed analysis of specific genes controlling Al resistance in *Brachiaria* and related species will increase our understanding of the underlying evolutionary molecular genetics and the interaction of Al resistance mechanisms.

Detailed report on activities of Output 4 is provided as Annex-4.

Output 5: *Capacity of students, NARS researchers and farming communities enhanced in stress physiology, functional genomics, molecular breeding, and participatory research and development (with special focus on participatory variety selection)*

The project staff members have trained 2 Postdoctoral fellows, 3 PhD students, 3 MSc students, 8 BSc students. Out of these 16 students, 4 students are women.

At the Leibniz University Hannover, Germany, 1 Postdoctoral Fellow (Dejene Eticha, Ethiopian) worked on interaction between Al and drought stress and proteomic aspects in common bean, 1 Ph. D. student (Zhongbao Yang, Chinese) is finishing his work on interaction of drought and aluminum toxicity stress in common bean and 1 BSc student (Caroline Quignon, German)

completed her work on Al resistance in *Brachiaria* and another BSc student (Nadine Heinze, German) completed her work on Al resistance in common bean.
At CIAT, 1 MSc student (Martin Emilio Rodriguez, Colombian) completed his work on gene expression and Al resistance in *Brachiaria*; and 3 BSc students in practical training (Fredy Orlando Ibanez Morales, Luisa Fernanda Vanegas Tombe, Daniel Adolfo Gomez Rodríguez**)** completed their work on screening for Al resistance in common bean. Andres Felipe Rangel, Colombian, completed PhD studies in Germany and conducted his Postdoctoral research at CIAT on developing phenotyping protocols for evaluating individual and combined stress factors of drought and Al toxicity.
The leader of the Rwandan national bean program (ISAR), Louis Butare is about to complete his PhD degree at University of Gembloux, Belgium, by conducting field, greenhouse and laboratory research at CIAT looking at physiological and genetic aspects of interspecific crosses of common bean. Domotille Mukukabanda (ISAR staff member), completed her BSc thesis work on farmer organization analysis at watershed level. Another ISAR staff member, Mupenzi Mutimura (Rwandan) has completed his MSc degree on participatory evaluation of *Brachiaria* grasses at the South African University of Kwazulu-Natal.
One MSc student, Mr. Bello Shano, registered with Bunda College of Agriculture, University of Malawi, finished course work and research, and has produced the first draft of his MSc thesis.
A training workshop on PVS organized by Dr. Louise Sperling was held in Mponela, Malawi from 28 to 30 May, 2008. Three project staff members from Malawi attended the workshop. It had 28 participants (20 male and 8 female).
A training of trainers' workshop on participatory plant breeding/PVS organized by Dr. Louise Sperling was held in Nazret, Ethiopia from 22 to 26 September, 2008. Four project staff members from Rwanda and Malawi attended the workshop. It had 23 participants (18 male and 5 female).
In Rwanda, a workshop with farmers was held to determine the best bet *Brachiaria* grasses accepted by farmers in two districts (Bugasera and Nyamagabe). Further training of 22 technicians on improved forage production and utilisation was conducted at ISAR Rubona Station in February 2010.
A second project workshop was held at Lilongwe, Malawi from 2 to 5 February 2009 to review the progress and to plan activities during the no cost extension period of the project (1 April 2009 to 31 March 2010). The workshop was attended by 12 scientists (3 from CIAT-Colombia, 3 from CIAT-Africa, 3 from ISAR-Rwanda, 2 from DARS-Malawi and 1 from Leibniz University Hannover, Germany).

9. Assessment of Research Findings

PVS on beans in Rwanda: The release of new drought resistant bean varieties was a break-through for ISAR-Rwanda as an institution. They will be utilized by the farmers in the drought prone eastern Rwanda to improve productivity and food security. The new bean varieties are already on high demand among the seed producers and traders. It is also important for the policy makers and the Ministry of Agriculture of Rwanda that are implementing the agricultural policies under the current Crop Intensification Program as well as food security targets for "Vision 2020" and the Millennium Challenge Goals.
PVS on beans in Malawi: Two bean genotypes, VTTT925/9-1-2 and BF 13572-5, that were selected by farmers showed have high yield potential under stress conditions and are recommended for release to farmers. SER 83 had highest yield under aluminum toxicity and drought stresses, the farmers also liked the variety for market.
PVS on Brachiaria in Rwanda: The important role of women in livestock rearing ought to be noted, although – for cultural reasons in Rwanda – they are not allowed to milk cows. Nevertheless, they share most activities with men. Interestingly, also women-headed households

appeared to put more efforts into innovative technologies than men, such as the introduction of new forage species.

The high productivity of improved *Brachiaria* cultivars and hybrids under environmental stress conditions (drought and acidic soils) together with their superior nutritional quality (crude protein content) make them very attractive alternatives for smallholder farmers who mostly rely on productive but lower quality Napier grass and some other forages in zero grazing systems. The rapid expansion of the area planted to these *Brachiaria* grasses shows that farmers have recognized their advantages over the traditional forages.

PVS on *Brachiaria* in Nicaragua: The good performance of the recently introduced commercial *Brachiaria* hybrid CIAT 36087 (cv. Mulato II) both during the wet and dry season and its good acceptance by farmers, together with its recognized drought tolerance, makes it an interesting forage option that could contribute to overcoming the constraint of lack of feed in the dry season. However, the high seed cost is a constraint to its dissemination among small scale farmers. It is therefore necessary to design strategies to increase seed availability and/or develop new hybrids with greater seed production potential.

Physiological and molecular mechanisms: New knowledge generated from this project has significantly contributed to the understanding of the physiological and molecular mechanisms of Al resistance and the interaction of osmotic/drought stress in common bean. It is expected that the identified genes will contribute to marker assisted selection and breeding of common bean cultivars adapted to acid soil where Al toxicity and drought are major yield limiting factors.

Phenotypic evaluation for individual and combined stress factors of drought and aluminum toxic acid soil: Phenotyping protocols have been developed and implemented to evaluate adaptation to individual and combined stress factors of drought and Al-toxic acid soils to support the on-going breeding programs of common bean and *Brachiaria*. Phenotyping methods used with hydroponics and soil tubes were found to be very complementary to field evaluations. Hydroponic method contributed to evaluate Al resistance while the soil tube method helped to assess the role of deep rooting ability in drought resistance, Al-toxic acid soil tolerance and tolerance to combined stress factors of drought and acid soil.

Development of genomic tools: A targeted gene approach allowed us to identify genes associated with physiological and molecular mechanisms such as release of organic acids from roots in response to Al toxicity in beans and *Brachiaria*. These genes will further contribute to understanding the resistance mechanism in crops through molecular and genetic studies. More than 1,000 clones from bean and *Brachiaria* treated with both drought stress and Al toxicity were identified from unique genetic resources that were never used in this type of work. These genomic tools including the differentially expressed genes using the gene pools will be used to create high density maps for bean and *Brachiaria* and to identify major QTLs for the key traits.

Development of bean lines: Lines combining resistance to two abiotic stresses are a unique output from this project. Although interspecific crosses typically do not result in improved lines in the short term, the cross of SER 16 with *P. coccineus* accession G35346 produced lines superior to the common bean recurrent parent. This effort was more productive than a broader intraspecific crossing program among common bean lines in a previous project on common bean funded by BMZ-GTZ (No. 2002.7860.6-001.00). The reaction of G35346-3Q is unique insomuch as some of its root parameters seem to respond favorably to aluminum toxic conditions. The release of drought resistant varieties in Rwanda is a first in Africa and bodes well for the release of improved drought resistant lines in other countries.

10. Knowledge Transfer

The improved bean varieties from the project were released in Rwanda on a well organized field day ceremony, in which ISAR scientists, government and policy makers, farmers, farmers' organizations and cooperatives, NGOs, donors and international partners (including CIAT), local and international press and e-media among others participated. The occasion created large

initial awareness about the new varieties. The demand for seed is overwhelming since the official release.

There has been much publicity of the new varieties in the local and regional media, including radio and TV. This awareness will be accompanied by mass production and distribution of existing brochures and posters depicting technical information about the varieties. A documentary film of 30 minutes was made to show the research and release, potential impact and way forward to access and utilize the varieties. This will be aired more frequently to raise more awareness and the dissemination strategy. Availability of seed will be crucial, and efforts will be made to multiply large quantities of breeder and pre-basic seed of the varieties to inject into the seed production chain.

Transfer of knowledge on improved forages in Rwanda has taken place via different training events (see below) and also by participatory assessments and selections. Results from the research have been presented during the 44th Annual Congress of the Grassland Society of Southern Africa (GSSA). Two scientific articles are being prepared based on the results from the MSc thesis.

Transference of knowledge and dissemination of *Brachiaria* hybrid CIAT 36087 (cv. Mulato II) in Nicaragua has only taken place in PVS activities and some training events with INTA technicians. There is still a need to validate the material by means of demonstration plots with farmers.

At the end of the project a workshop on "Dry season feeding – problem at present and challenge for the future" was organized at national level with some 60 farmers, technicians, researchers (INTA, CIAT, UNA-National Agricultural University, and others) and representatives of farmer organizations. The objectives of this workshop were: (i) presentation of project results; (ii) presentation of experiences in dry season feeding in Nicaragua (last 4 years); and (iii) discussion of results, formulation of a strategy for the coming years. This workshop facilitated knowledge transfer on the following themes: (i) constraints and opportunities for improving forage production; (ii) potential contribution of drought adapted forages: both grasses (with a focus on *Brachiaria*) and legumes (use of legumes in combination with crop residues, leguminous trees/shrubs in agroforestry systems) to maintain/increase animal production in the dry season and/or increase soil fertility; (iii) conservation of forages (silage, hay); and different climate change scenarios and their effects on forage production.

The results of this research project on physiological and molecular aspects and the methodological approaches applied for the study were published in international journals and communicated at national and international conferences. The project is the basis of an ongoing scientific cooperation with Jilin University, China, with exchange of scientists and students in the frame of a DAAD-funded project. Gene sequences generated from this project are being deposited in public domain for free access.

Project results on bean improvement were shared in international forums in Australia, China, Mexico and the United States. Two research papers have been submitted on work with common bean. Interspecific lines were shared with the Ethiopian national bean research program for testing at an acid soil site. Lines were also shared with the researchers in Kenya and Uganda for evaluation for resistance to biotic constraints.

11. Training

The project staff members have trained 2 Postdoctoral fellows, 3 PhD students, 3 MSc students, 8 BSc students. Out of these 16 students, 4 students are women.

In order to transfer skills and capacity for modern technology of bean production, 150 farmers from Nyagatare, Gatsibo and Kabarole of Umutara project area were trained on production of good quality seed and on how to identify and manage key bean diseases and pests, and soil related constraints. They were also trained on the importance of planting good seed of improved varieties, soil fertility and good husbandry practices of beans in the field and at post-harvest.

Workshops have been organized, at least once at the beginning of a season. These were initiated by ISAR, Rwanda or the other partners. Joint action plans were usually made and monitored. The most recent was a regional workshop organized by PABRA in Bujumbura, Burundi (for DR Congo, Burundi, Kenya, Uganda and Rwanda), where all major national partners in research and in seed multiplication participated, shared experiences and developed a seed multiplication and variety dissemination plan of action.
Field staff and scientists were trained in participatory research approaches for forages. A 10-day training workshop took place in October 2006 at ISAR-Rubona, in order to equip researchers and field workers with approaches for participatory forage evaluation and forage agronomy. During the workshop, 15 participants from wards and NGOs of Bugesera and Nyamagabe districts shared knowledge of forage agronomy with smallholder farmers. A 5-day training workshop on "Improved forage production and utilisation in Rwanda" was conducted at ISAR-Rubona in February 2010 for instructing overall 22 technicians and extension workers from NARS and NGOs in the domain of forage and animal feeding in Rwanda. Participants came from all regions of Rwanda, which will improve knowledge transfer within the country. The course topics included the role of improved forages, important characteristics of forages, growth and adaptation of forages, participatory diagnosis, and animal feeds and feeding. Post-graduate training of an ISAR forage scientist (Mupenzi Mutimura, ISAR-Rwanda) took place both during *Brachiaria* on-farm evaluation activities in Rwanda and at University of Kwazulu-Natal, Pietermaritzburg, South Africa. Two technicians of both the regional INTA-Nicaragua office Pacifico Sur and Headquarters received training on participatory research approaches for forages during the PVS events. This also enabled them to evaluate the performance of the new *Brachiaria* hybrids and cultivars in the field experiment.

12. Lessons Learned

The farmers' ranking of the same set of varieties can change depending on time of evaluation; season and environment. Just as formal breeders, farmers need to be able to evaluate the varieties at different stages and over several seasons. "Secret balloting" / placement of ribbons in envelopes; different colors of ribbons used in selection for men and women, and excellent questioning (probing) skills during discussions were key components of a successful PVS exercise. Traders evaluating in the Malawi sites also added a quick 'marketing check' on the possible 'saleability' of the proffered varieties. Selection for Al toxicity was a bit challenging; a more refined protocol is needed in future.
Already after short exposure to them, farmers selected for multiplication those grasses that were highly productive throughout the year. They were apparently convinced of their adaptability to drought and acidic soil stress conditions. The five grasses established for multiplication by farmers were cv. Mulato II, cv. Toledo, cv. Marandu, *Brachiaria* hybrid BRO2/1485 and cv. Basilisk. The same experimenting farmers expanded their areas with improved grasses, while other farmers also began to establish them. Interestingly, farmers in the two districts formed specific cooperatives for *Brachiaria* multiplication. This demonstrates (i) the importance of a thorough problem assessment before initiating the research, and (ii) the value of participatory selection on farm, while considering gender aspects. Evaluation of adaptation of *Brachiaria* grasses in Nicaragua indicated that higher seed availability of each material would have allowed multiplication in a nursery for replanting after failure. Besides, a closer supervision by a technician constantly interacting with the farmers would have led to a closer management and monitoring of the trials and a more active participation in PVS events. A more refined protocol for PVS events is necessary to define the key moments to conduct PVS and to define more clearly the selection criteria and their assessment, as well as the different types of farmers and other stakeholders.
Studying a plant species (common bean) which is molecularly difficult to work with because of lack of genomic information is complicated and time consuming. In depth physiological

characterisation is a prerequisite for adequate exploitation of the molecular information. Thus the initiated and conducted extensive proteomic and transcriptomic approaches need follow up studies for full validation.

Three major lessons were learned from phenotypic screening. First, resistance to Al at root level need to be combined with photosynthate mobilization to grain for improving acid soil tolerance and deep rooting ability should be combined with photosynthate mobilization to improve drought resistance. Second, pod harvest index in common bean could be a very useful trait for both individual and combined stress factors of drought and Al-toxic acid soils. Third, fine root development is an important trait for individual and combined resistance to acid soil and drought in *Brachiaria*.

For common bean improvement, drought resistance as assessed in Colombia under managed drought conditions has been found to be useful in production areas in Rwanda. *P. coccineus* is an excellent source of Al resistance to improve common bean. Its root system appears to be very unlike that of *P. vulgaris*, and probably reflects very different evolutionary pressures. *P. coccineus* should be investigated as a source of traits for other edaphic constraints. Poor harvest index is a major problem of progenies of crosses between common bean and the secondary gene pool. Crossing with a common bean such as SER 16 with enhanced remobilization of photosynthate to grain improved the value of progenies from these crosses by channeling more biomass to grain. This may be a key to tapping the diversity of the secondary gene pool which to date has scarcely been touched for the improvement of common bean. The soil tube method yields results that correlate with field trials in acid soil, but the relevance of results with the solution culture to field performance is not clear. Very few traits measured in the two systems correlate with each other.

13. Future Research Needs

There are relatively very few scientists working on combined abiotic stress tolerance in common bean and *Brachiaria*. This project has been extremely productive in creating interest and strengthening capacity of NARS programs for developing beans and *Brachiaria* with resistance to drought and Al toxicity. The team in Hannover, Germany contributed to advanced research on physiological and molecular mechanisms of abiotic stress resistance of common bean. At the institutional level, ISAR-Rwanda needs to establish capacity to carry out physiological and molecular genetic studies on drought and Al toxicity, as most of the research was dependent on phenotypical and farmers' assessments. There is need to continue selection for the same constraints among newly generated RIL populations. There could be need to test whether experienced breeders who have worked with farmers can be sufficiently competent to select farmers' varieties in a more cost-effective manner. There should be great effort for massive seed production and dissemination of the drought tolerant bean varieties to farmers.

There is also need to go along with farmers while they are adopting the improved *Brachiaria* grasses. It is necessary to monitor herbage quality over longer time, particularly if no fertilizer is applied. However, with higher livestock productivity also more manure will be produced that should be recycled to the forages. Hence, nutrient cycling together with nutritional quality of forage and milk and animal health will need to be assessed over a period of time to document the sustainability of the improved production system. Provisions need to be made to release any *Brachiaria* hybrid that has not yet been named properly as farmers will spread these materials rapidly if they hold what they promise. However, a proper cultivar name would facilitate such dissemination.

The project activities in Nicaragua led to the farmers' knowledge, evaluation and selection of a promising drought tolerant hybrid (cv. Mulato II), but experience has shown that management recommendations are also needed to ensure a lasting performance. It is also necessary to evaluate the performance (dry matter yield, persistence, effect on animal production) of this material under different soil and rainfall conditions, by means of validation trials, and including

different forms of management considering factors like stocking rate, grazing pressure and fertilizer application.

Since genotypic Al resistance in common bean specifically depends on the capacity to sustain the synthesis of citrate for maintaining the cytosolic citrate pool that enables exudation, the characterisation of theses genes is a present research focus. In addition, the full cDNA sequence of the identified citrate transporter genes and their functional characterisation needs to be undertaken. Also, the present research focuses on the identification of drought and osmotic stress-specific proteins and genes evaluating 2D IEF/SDS PAGE proteomics, phospho-proteomics and transcriptomics (SuperSAGE). Expression of the identified genes will then be studied in root tips grown soil subjected to soil acidity (Al) stress, drought stress and a combination of both stresses.

The identified genes should be further mapped in the *Brachiaria* and bean genomes to create high density maps to accelerate QTL cloning for the traits of interest. The mapping and QTL cloning can be accelerated by using close species genome sequences such as those from soybean and *Brachypodium* (for bean and *Brachiaria*, respectively) that were recently released. For bean, there is an ongoing initiative to sequence the common bean genome in the USA, which will be facilitating our molecular breeding in the future.

Further work is needed to characterize the mechanisms of photosynthate transport during individual and combined stress conditions of drought and acid soil stress and to identify the major genes responsible for this physiological mechanism in common bean.

The root phenotypes obtained from the interspecific cross need to be characterized for their value in different soil environments. Interspecific sister lines with different root traits should be intercrossed to recover more of the coccineus phenotype. *P. coccineus* accession G35346-3Q should be studied more intensively to characterize its aluminum tolerance and its unusual response to aluminum toxicity. The secondary gene pool of common bean including *P. coccineus*, *P. dumosus*, and *P. costaricensis* should be examined more extensively for resistance to both abiotic and biotic constraints, with the perspective of employing them more widely and effectively in crosses with improved common bean genotypes such as SER 16. Additional strategies may need to be developed to successfully introgress traits from *P. coccineus* into common bean of the Andean gene pool.

Further research is also needed to characterize the mechanisms of drought resistance in *Brachiaria* and to identify the major genes responsible for its adaptation to combined stress conditions of drought and acid soil. It is also important to continue with the saturation of the *Brachiaria* linkage map to increase the power of detection of genome regions that govern the quantitative characteristics of individual and combined resistance to drought and aluminum stress. Additional investigations on physiological and molecular mechanisms, interactions between different metabolic pathways, systems biology and a detailed analysis of specific genes controlling Al and drought resistance in *Brachiaria* and related species will increase our understanding of the evolutionary molecular genetics and stress resistance mechanisms.

14. Summary

The project has made several outstanding contributions for genetic improvement of common bean and *Brachiaria*. The ISAR-Rwanda bean program accomplished the following: (i) released two drought tolerant semi-climbing bean varieties, SER 16 and SER 30 of farmer preferred small red seed market with a yield potential of 2.0 to 2.5 t/ha; (ii) released 3 heat and drought tolerant climbing bean varieties adapted to low altitude and warmer zones of eastern Rwanda, MAC 9, MAC 44, and MAC 49 with potential yield of 3 to 3.5 ton/ha, (in collaboration with other breeding projects by AGRA and Pulses CRSP); (iii) identified pre-release drought tolerant bush and semi-climbing bean varieties for the semi-arid zones of eastern Rwanda Bugesera and Umutara: SER 12, SER 13, SER 14, NR1467-17-6P, MR14258-7-8P, MR14265-58-2P and TROCALENA75 that were selected for superior yield and earlier maturity compared to the released and local

checks; (iv) identified and selected advanced Al toxicity tolerant bean lines from recombinant inbred lines (RILs) for drought and/or Al toxicity potential for adaption in the Al and acid stress conditions in Gikongoro/Nyamagabe in southern Rwanda. The lines CIM-RMOO 323LNO2, CIM-RMOO 321 LNO3, VTTT924/2/2-4-2-1, SEN 13, SEN 23, SEN 24, ENTRY 18, and ENTRY 20 are the most promising lines among the test entries for Al toxicity tolerance; (v) gained and practiced skills for conducting a successful farmer participatory evaluation exercise through PVS training; (vi) documented criteria and preferences used by farmers, traders and consumers in the selection of bean varieties in the drought-prone environments through farmer participatory evaluation approaches; (vii) developed human capacity and skills in the form of higher degree training (PhD); and (viii) initiated and catalyzed breeder and pre-basic seed production and develop linkages with other actors (RADA, NGOs, Private farmers, Farmers Cooperatives) and stakeholders in the research and extension services for seed multiplication and variety dissemination of the new drought tolerant varieties through workshops.

ISAR-Rwanda-Forages Program conducted research for development work in two districts with contrasting environments, drought-prone (Bugesera district) and acidic soils (Nyamagabe district). Participatory assessments of feed resources and participatory selections were performed with local farmers. Three cultivars and five hybrids of *Brachiaria* grass from CIAT and two local grasses (control) were used for small-plot on-farm participatory trials. Twelve farmers each collaborated in the two study areas. The high productivity throughout the year of improved *Brachiaria* grasses under the given environmental stress conditions together with their superior nutritional quality (crude protein content) make them very serious alternatives for smallholder farmers for zero grazing systems. The rapid expansion of the area planted to these *Brachiaria* grasses shows that farmers have captured their advantages over the traditional forages. In addition, training on forages, feeding and participatory research approaches performed at different levels (farmers, technicians and extension workers, academic) will assist knowledge transfer within the country.

Physiological studies conducted by the team in Hannover, Germany demonstrated that the aluminium resistance in common bean requires both, Al-induced exudation of citrate from the root apices and the maintenance of cytosolic citrate concentrations through enhanced citrate synthesis. The exudation of citrate requires the up-regulation of MATE genes coding for citrate transport proteins. The expressions of genes encoding enzymes involved in citrate metabolism were not significantly affected by Al suggesting posttranslational regulation. Therefore, high Al resistance of common bean primarily depends on citrate synthesis which should be the focus of future studies. The team in Germany also assessed the genotypic differences in osmotic/drought stress tolerance using PEG in hydroponics. PEG stress completely relieved Al stress by strongly reducing Al uptake and binding in the root apices. An in-depth study provided circumstantial evidence that the osmotic stress-inhibited Al accumulation in root apices and thus reduced Al-induced inhibition of root elongation in the Al-sensitive genotype VAX 1 is related to the alteration of CW porosity resulting from PEG 6000-induced dehydration of the root apoplast. The identification of transcripts typically regulated by osmotic/drought stress will allow the study of the Al/drought interaction in root tips subjected to Al and drought stress in an acid soil.

Molecular studies conducted by the team in Germany indicated that Al treatment dramatically increased the expression of common bean expressed sequence tags belonging to the citrate transporter gene family *MATE* (multidrug and toxin extrusion family protein) in both the Al-resistant and sensitive genotype in close agreement with Al-induced citrate exudation. From this it is concluded that the expression of a citrate transporter *MATE* gene is crucial for citrate exudation in common bean. However, although the expression of the citrate transporter is a prerequisite for citrate exudation, genotypic Al resistance in common bean particularly depends on the capacity to sustain the synthesis of citrate for maintaining the cytosolic citrate pool that enables exudation.

Molecular work conducted by the team at CIAT led to the identification of major genes associated with Al resistance such as *STOP* and *ALMT1* in beans and *Brachiaria* in addition to *MATE* mentioned above. These genes were previously reported as major genes for Al resistance in other crops such as wheat. Moreover, the position of the candidate genes for Al-resistance was predicted on *P. vulgaris* chromosome using the synteny between soybean and common bean. Some of the candidate genes were matched to the postion of QTL for Al-resistance. These findings need to be further confirmed through genetic mapping. For *Brachiaria*, although homologous genes of *ALMT1, STOP1* and *MATE* were identified in *Brachiaria* we cannot conclude that these genes are associated with Al resistance based on the gene expression pattern.

In addition to the targeted approach described above, some candidate genes to be involved in drought and Al resistance in beans were isolated by constructing subtractive cDNA libraries. As a result of the subtractive screening, we have isolated one and three up-regulated genes in drought and Al resistant genotypes, respectively, using contrasting genetic materials (for drought, a drought resistant genotype, G 40159 (*Phaseolus acutifolius*) and a drought sensitive genotype, DOR 364 (*Phaseolus vulgaris*): for Al resistance G 35346-3Q (*Phaseolus coccineus*), an Al-resistant genotype, and SER 16 (*Phaseolus vulgaris*), an Al sensitive genotype).

Phenotyping protocols have been developed and implemented to evaluate adaptation to individual and combined stress factors of drought and Al toxic acid soils to support the on-going breeding programs of common bean and *Brachiaria*. Phenotyping methods used with hydroponics and soil tubes were found to be very complementary to field evaluations. Hydroponic method contributed to evaluate Al resistance while soil tube method helped to assess the role of deep rooting ability in drought resistance, Al-toxic acid soil tolerance and tolerance to combined stress factors of drought and acid soil.

Common bean lines combining resistance to two abiotic stresses are a unique output from this project. The cross of SER 16 with *P. coccineus* accession G35346 produced lines superior to the common bean recurrent parent. The reaction of G35346-3Q is unique insomuch as some of its root parameters seem to respond favorably to aluminum toxic conditions. The release of drought resistant varieties in Rwanda is a first in Africa and bodes well for the release of improved drought resistant lines in other countries.

A new genetic linkage map was constructed for *Brachiaria decumbens,* (using a hybrid population generated from a cross of two *Brachiaria* parents) with eighteen linkage groups that covered 1094 cM (mean 60.7 Cm and 6.7 markers per group) and 122 molecular markers including SSR, EST, AFLP and SCARs. Five putative QTLs were found in five different linkage groups (LG1, LG3, LG4, LG7 and LG17). These QTLs explained the 6.6%, 7.8%, 11%, 13% and 21% of the aluminum stress response, respectively. It is necessary to continue with the saturation of the linkage map to increase the power of detection of genome regions that govern the quantitative characteristics of Al resistance in *Brachiaria*.

The 79 most promising *Brachiaria* hybrids developed by the breeding program for the past 6 years were evaluated for their resistance to Al in nutrient solution and 7 hybrids (BR04/02069, BR05/00334, BR05/00563, BR06/0012, BR06/0531, BR06/1175, BR06/1278) were identified with high root vigor and higher level of Al resistance. These hybrids also combine some level of resistance to major insects called spittlebugs and therefore could be useful for integration into crop-livestock systems in the tropics.

15. Publications, Papers and Reports

The project staff members have published a total of 21 journal articles, 2 book chapters, 6 articles in conference proceedings and 37 oral and poster presentations at international and national conferences and workshops. A total of 10 students (2 PhD, 3 MSc, 5 BSc) theses were approved by different universities in Germany, Rwanda, Malawi and Colombia.

A list of publications, papers, reports and theses is provided as Annex-5.

ANNEX 1

Project Title

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

Project Supported by
Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)

Executed by
International Center for Tropical Agriculture (CIAT)
in collaboration with
University of Hannover, Germany
Institut des Sciences Agronomiques du Rwanda (ISAR), Rwanda
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Instituto Nicaragüense de Tecnología Agropecuaria (INTA), Nicaragua

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Participatory evaluation of common bean and Brachiaria

ANNEX-1

Participatory evaluation of common bean and *Brachiaria*

Output 1: *Rural benefits enhanced in the target areas of tropical Africa and Central America by involving women and small-scale farmers as decision makers and co-researchers in the common bean and Brachiaria product development process*

ISAR-Bean Program, Rwanda

Contributors: A. Musoni, F. Nsanzabera, D. Maukukabanda and V. Ruganza

Introduction: Common bean (*Phaseolus vulgaris*) is the most consumed food legume and crucial source of protein and micronutrient nutrition, especially among the rural poor, women and children in Rwanda. Beans are produced on small land plots that aggregate to about 300,000 ha (about 30% of cultivated land) in Rwanda. More than 99% of the bean acreage is rain fed.

The development and soil penetration of root systems is critical in water and nutrient absorption capacity, especially when soil moisture is limiting (drought). Drought ranks top among abiotic and biotic bean production constraints in sub-Saharan Africa (Wortmann, 1988). In Rwanda, drought stress was the most important cause of low productivity and reported famines in Mayaga, Bugesera and Umutara agro-ecological zones to the East, where rains are scanty and/or sporadic. It is exacerbated by prevailing higher temperatures.

Associated with high acid sub-soils (pH of 4.0 $\leq$ 5.50), aluminum (Al) toxicity, is an acute bean production problem in southern Rwanda, particularly in former Gikongoro province and current districts of Nyamagabe and Gisagara. Al toxicity hampers root growth, elongation and extensive sub-soil penetration. The availability and uptake of P is particularly limited in acid soils. Al toxicity, therefore, complexes and exacerbates drought.

In Rwanda, and at the global level, the economic loss caused by drought and / or Al toxicity is expected to rise due to land pressure and climatic change and human activities in marginal lands.

Activities and role of ISAR bean program: The main role of bean breeding program in Rwanda (ISAR) was to carry out participatory variety evaluation and selection of drought and Al toxicity resistant bean varieties in drought and Al toxicity hot spot sites at Bugesera, Karama, Umutara,Nyagatare for drought; and Gikongoro / Nyamagabe for aluminum toxicity.

In summary, within the 3 years of the project, ISAR bean breeding program was able to accomplish the following:

1. To release two drought tolerant semi-climbing bean varieties, **SER 16** and **SER 30** of farmer preferred small red seed market with a yield potential of 2.0 to 2.5 ton / ha.
2. To release 3 heat and drought tolerant climbing bean varieties adapted to low altitude and warmer zones of eastern Rwanda, **MAC 9, MAC 44,** and **MAC 49** with potential yield of 3 to 3.5 ton / ha, (in collaboration with other breeding projects by AGRA and Pulses CRSP).
3. To identify pre-release drought tolerant bush and semi-climbing bean varieties for the semi-arid zones of eastern Rwanda Bugesera and Umutara: **SER 12, SER 13, SER 14, NR1467-17-6P, MR14258-7-8P, MR14265-58-2P** and **TIOCANELA75** that were selected for superior yield and earlier maturity compared to the released and local checks.
4. To identify and select advanced Al toxicity tolerant bean lines from recombinant inbred lines (RILs) for drought and / or Al toxicity potential for adaption in the Al and acid stress conditions in Gikongoro/Nyamagabe in southern Rwanda. The lines **CIM-RMOO 323LNO2, CIM-RMOO 321 LNO3, VTTT924/2/2-4-2-1, SEN 13, SEN 23, SEN 24, ENTRY 18**, and **ENTRY 20** are the most promising lines among the test entries for Al toxicity tolerance.
5. To gain and practice skills for conducting a successful farmer participatory evaluation exercise through PVS training.
6. To document criteria and preferences used by farmers, traders and consumers in the selection of bean varieties in the drought-prone environments through farmer participatory evaluation approaches.
7. To develop human capacity and skills in the form of higher degree training (PhD) (to be reported elsewhere).
8. To initiate and catalyze breeder and pre-basic seed production and develop linkages with other actors (RADA, NGOs, Private farmers, Farmers Cooperatives) and stakeholders in the research

and extension services for seed multiplication and variety dissemination of the new drought tolerant varieties through workshops.

9. Published one local student (BSc) thesis and written a draft journal publication on climbing beans adapted to low altitude elevations in Rwanda.

1. Participatory evaluation of newly introduced lines for Al toxicity resistance at Gikongoro

During 2008 B, a trial was established to evaluate 19 previously selected RILs that were introduced from CIAT for Al toxicity tolerance at Gikongoro. Two ISAR varieties: RWR 1668 (released variety) and MLB 49-89 A were included as checks to make a total of 21 entries. 40 healthy seeds of each entry were planted in two 2-m rows with 50 cm and 10 cm between and within rows respectively, to make a plot area of 4 m^2 in two separate blocks (replications). The first block was uniformly treated with lime at 2.5 ton / ha to counteract the high acidity (pH 4.5) and Al toxicity. It was labeled as "Non-stress condition" (NSC). The second block was not treated with lime so as to maintain high acidity levels. It was labeled as "Stress condition" (SC). The 2 checks were planted at intervals after every 4 entries. Data was recorded on prevalent diseases incidence and severity on a CIAT scale of 1 – 9; on plant stand at germination and harvest. The latter was used to estimate plant survival rate as percentage of harvested plants to the seed rate for each entry. Plot yield in grams was measured and was converted to yield in kg / ha. The results were summarized in Table 1.

From the Table 1 in appendix 1, mean yields and survival rates were higher (702 kg/ha and 74%) under non-stress conditions than in case of the stressed conditions (232 kg/ha and 61% survival), meaning high acidity associated with Al toxicity affected productivity of the test RIBLs entries as well.

When the overall survival means of the test entries and checks were compared, it was found that the more adapted checks had the highest rates of survival than the test entries (Figure 1) (probably due the low number). The overall mean for survival among the test entries under stressed and non-stressed conditions were not different, meaning the variability for tolerance to acid soils was high (with normal distribution) among the entries. This is further confirmed when some of the individual test entries had both higher survival rates and yields than the checks (Figures 2 and 3).

The lines **CIM-RMOO 323LNO2, CIM-RMOO 321 LNO3, VTTT924/2/2-4-2-1, SEN 13, SEN 23, SEN 24, ENTRY 18**, and **ENTRY 20** are the most promising lines among the test entries for Al toxicity tolerance.

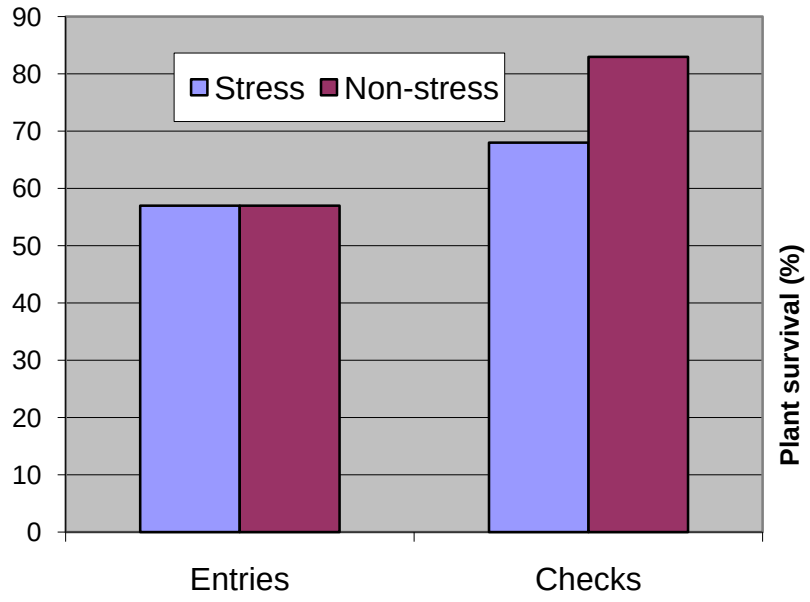

Figure 1. Means of plant survival among Al tolerant recombinant inbred lines (RIBLs) and local checks under stress and non-stress conditions at Gikongoro 2008B season.

Table 1. Diseases reaction, yield and plant survival of recombinant inbred lines for Al toxicity evaluated at Gikongoro in 2008B. Checks are in bold.

Variety	RUST	BCMV	ASCO	ALS	ANTH	Seed size	Seed color	Yield in kg/ha (SC)	Yield in kg/ha (NSC)	Survival (%) NSC	Survival (%) SC
MLB49-89A	2	5	3	5	1	Small	black	300	450	85	60
RWR1668	2	2	3	4	1	Large	Dark red	300	600	75	75
ENTRY20	2	5	2	6	1	Large	Pink	450	900	73	70
ENTRY17	3	5	2	4	1	Large	Pink	0	600	33	33
SEN4	5	4	2	5	1	Small	black	0	0	50	20
MN14059-4-4P	3	4	2	6	1	Medium	Red	300	600	55	20
MLB49-89A	2	2	3	6	1	Medium	Black	600	750	78	80
RWR1668	3	2	2	4	1	Large	Dark red	300	450	90	88
ENTRY18	2	4	2	7	1	.?	?	0	750	80	70
ENTRY22	2	3	2	8	1	medium	White	300	600	83	60
VTTT924/2-4-2-1	2	3	2	4	1	Large	Red	300	0	78	70
CIM-RM00-323-O2	3	3	2	3	1	Large	Red /white	300	1350	95	88
MLB49-89A	3	3	2	5	1	S mall	Black	300	900	80	78
RWR1668	4	6	2	5	1	Large	Dark red	300	1050	98	63
ENTRY25	3	4	2	6	1	.?	?	0	450	45	45
ENTRY21	2	3	4	7	1	Medium	Pink	0	1050	88	60
SEN23	7	3	3	5	1	Small	Black	600	750	73	83
NCB275	3	5	2	7	1	?	?	150	450	65	58
MLB49-89A	2	5	4	5	1	Small	Black	150	1050	90	60
RWR1668	2	3	4	3	1	Large	Dark red	150	900	65	78
ENTRY30	4	3	3	7	1	Medium	Red	450	300	55	45
ENTRY24	5	3	3	7	1	Medium	black	0	450	65	38
CIM-RM00-321-O3	3	4	5	3	1	Large	Red/white	300	600	78	78
ENTRY23	2	6	3	5	1	Large	Red/white	150	0	48	48
MLB49-89A	2	4	3	6	1	Small	Black	150	600	83	45
RWR1668	2	3	2	3	1	Large	Dark red	300	900	83	55
SEN13	2	5	3	7	1	Medium	Pink	450	900	73	88
SER4	2	4	3	8	1	Medium	Red	300	1200	80	70
ENTRY32	8	5	2	6	1	.?	?	0	1500	88	38
MLB49-89A	3	3	2	6	1	Small	Black	150	900	90	83
RWR1668	3	2	2	4	1	Large	Dark red	150	750	80	48
Mean								232	702	74	61

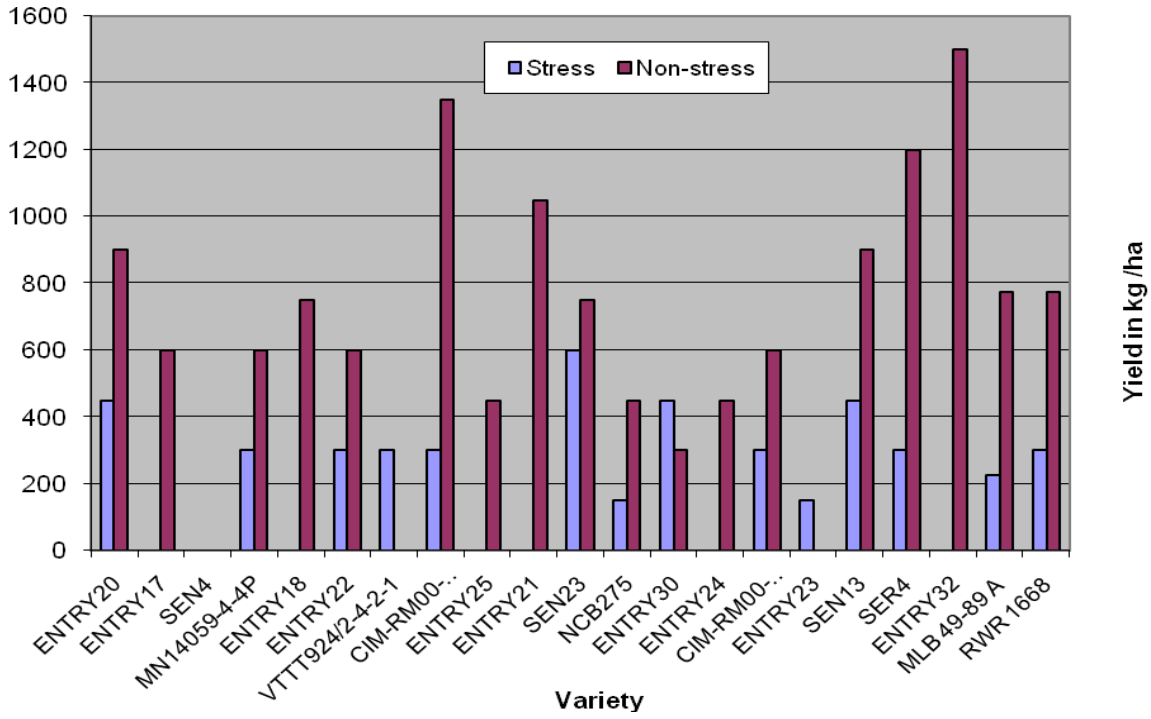

Figure 2. Yield of RIBLs for Al tolerance and local checks (MLB 49-89 A and RWR 1668 under stress and non-stress conditions at Gikongoro in 2008B season.

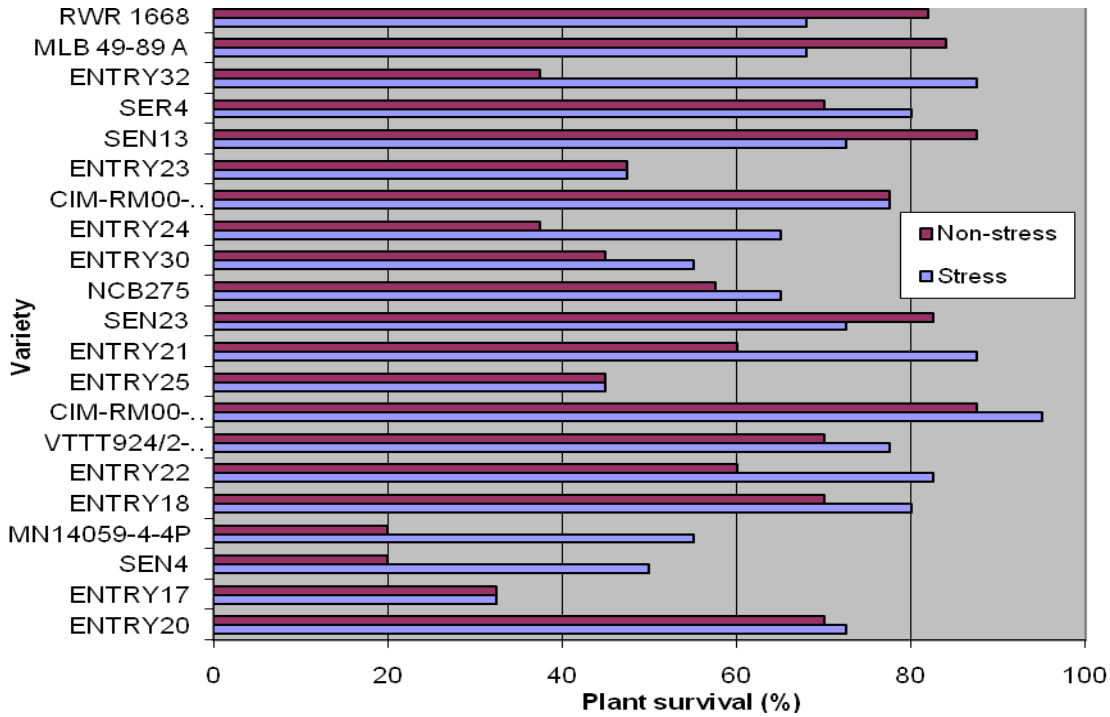

Figure 3. Varietal plant survival of RIBLs for Al tolerance and local checks (MLB 49-89 A and RWR 1668 under stress and non-stress conditions at Gikongoro in 2008B season.

2. On station and on farm testing and evaluation of introduced lines that combine drought with Al toxicity at Karama and Nyagatare

2.1. Participatory selection and release of drought tolerant bean varieties.

The specific objectives were to assess adaptation of locally adapted and introduced RIBLs for drought tolerance and acceptability and catalyze the adoption of the varieties by farmers. In 2006, thirty eight (38) drought tolerant SER and SEN lines were introduced from CIAT for participatory evaluation for adaptability and acceptability by farmers in the drought sites of Karama (representing Bugesera zone) and Nyagatare representing Umutara zone). Preliminary evaluation at both sites selected 14 lines SER 16, SER 14, SEN 23, SER 18, SEC 10, SER 12, SER 22, SEN 13, SEN 32, SEN 2, SEN 24, SEN 18 and SEN 31. At Nyagatare research station, 18 lines: SER 30, SEN 5, SER 5, SEN 31, SER 21, SER 16, SER 18, SEN 7, SEC 2, SEN 21, SEN 6, SER 12, SEC 11, SEN 26, SEN 4, SER 4 and SEC 17 were selected in the participatory preliminary trials.

Yield, early maturity, tolerance to diseases, vigour, root and stem firmness, green shoot system and leaves as opposed to wilted/dried shoot and leaf canopy were among the criteria that was used in the participatory selection processes. Their yield ranged from 1.4 – 3.0 t/ha and maturity was 75 – 85 days.

The selected varieties were then subjected to further multilocation trials over years and seasons in Bugesera and Umutara zones. In Bugesera, **SER 12** and **SER 14** were selected for pre-release in 2009 and are ear-marked for release in this year, 2010. The maximum yield recorded were 2.5 ton/ha and 2.3 ton/ha for SER 14 and SER 12 respectively. They both matured at 80 days. SER 12 was the more appreciated by farmers for the apparent early maturing, very short cycle, adapted to the region with short rains, vigorous strong stem that resists wind and logging, and good small red seed coat associated with good culinary and market qualities.

Multilocation testing and screening over years and seasons under participatory approaches resulted in the selection and release of **SER 16** and **SER 30** for the entire semi-arid zones in Nyagatare/Umutara zones and Bugesera (Figure 1). They averaged a yield potential of 2.0 – 2.5 ton / ha. Besides yield and drought tolerance traits, the varieties were appreciated by farmers for the early maturity, good taste, fast cooking and the fact that they do not stain source pans after cooking. Clear/shiny red seed coat colour was the inference for this, according to the farmers (Figure 2). The red seed colour was associated with red broth coloration imparted on tubers and cereals when cooked as mixtures (Agatogo). They were baptized **Munyu 1** (**SER 16**) and **Munyu 2** (for **SER 30**). Munyu means salty or good taste.

Figure 1. One of the drought resistant variety, SER 16 that was released by ISAR in Rwanda in January, 2010: in the field (left) and its grain (right).

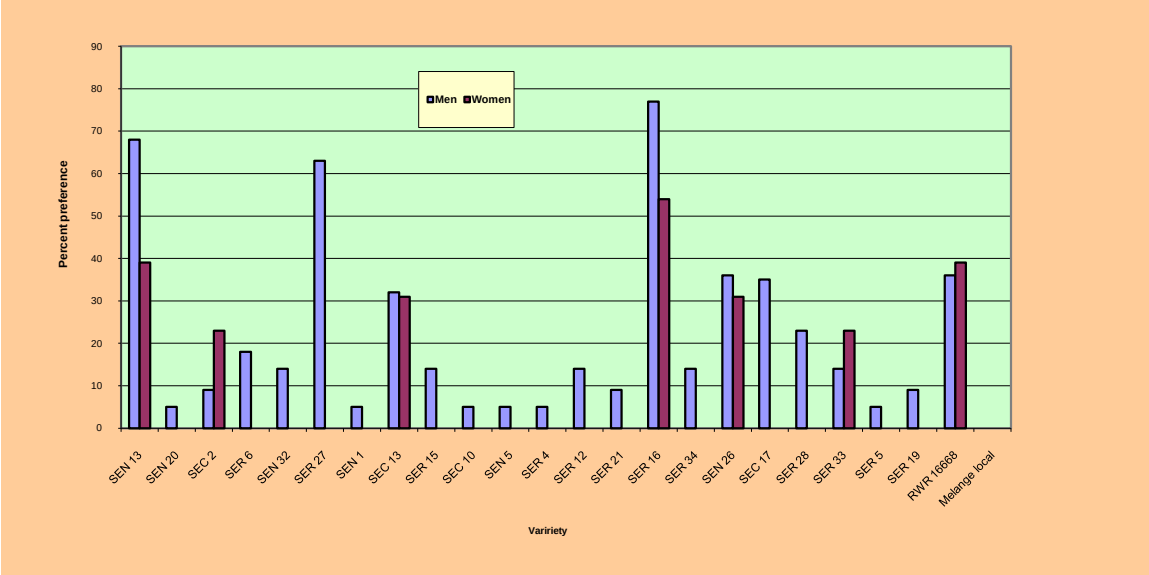

Figure 2. Aggregate farmer participatory selection of drought tolerant lines by men and women farmers at Nyagatare and Karama sites.

2.2 Adaptability trials for drought tolerance and early maturing bush lines

During the same period, participatory selection from advanced drought nursery that were introduced from CIAT continued. They were evaluated across locations in Karama and in Nyagatare zones with local checks. The results of yield, diseases reaction and maturity are indicated in Table 1 below.

Figure 3. Apparent variation in maturity duration among advanced bush and climbing bean lines (back ground) planted at ISAR Nyagatare research station. The earliest maturing bush line is arrowed in white, while the late local check bush line is red-arrowed; and the intermediate maturing line is arrowed yellow.

Among the advanced drought tolerant lines, **NR1467-17-6P, MR14258-7-8P and MR14265-58-2P** were selected for superior yield and earlier maturity compared to the released and local checks. The advanced line, **TIO CANELA 75** was among the highest yielding, but was rejected by farmers for the longer maturity duration. They will be tested in more locations in Nyagatare and Bugesera regions in the subsequent seasons and the most elite among them be characterized and recommended for release to farmers (Figure 3).

Table 1. Diseases reaction, yield and other agronomic data of the advanced drought tolerant bush bean lines trial at Cyabayaga ISAR station in Nyagatare District.

Variety	Germ ((%)	Vigour	Flowering (50%)	Maturity (50%)	CBB	BCMV	Rust	ALS	Asco	Pod /plant	Seed /pod	Yield (kg/ha)	% check^{+}	% check^{++}
MN13856-35-3P	51	3	49	81	4	3	2	1	2	31	7	1875	126	92
NR1467-17-6P	95	4	51	80	3	1	1	1	1	20	6	2167	145	107
MR14232-27-9P	81	4	50	81	3	1	1	1	1	15	6	1875	126	92
MR14258-7-8P	87	5	52	84	5	3	1	1	1	18	6	2292	154	112
MR14265-58-2P	79	6	50	82	3	2	1	1	1	17	6	2541	170	125
MR142116-6-6P	39	6	50	80	2	2	1	1	1	20	6	1958	132	96
MN14059-4-4P	88	5	51	80	2	1	1	1	1	19	6	1833	123	90
TIOCANELA75	88	5	50	86	5	3	1	1	1	18	5	2334	156	114
A774	36	3	50	78	4	2	1	1	1	29	6	1917	128	93
MR13937-27-4P	41	5	50	82	4	2	1	1	2	37	7	1750	117	86
MR140000-2-5P	87	4	50	81	5	2	1	1	1	11	5	1792	120	89
MN13946-10-7P	68	4	50	81	3	2	1	1	1	20	5	2041	137	100
NR14194/19-2P	87	4	50	84	3	2	1	3	3	24	7	2084	140	103
$^{++}$RWR 1668	87	4	50	82	2	2	2	1	1	21	7	2042	137	100
$^{+}$Local mixture	65	2	50	84	5	6	4	7	1	10	6	1500	100	76
Probability (5%)										*	NS	**		
C.V. (%)										22.3	12.9	23.1		

$^{+}$ = Local farmers variety (mixture) check; $^{++}$ = Improved ISARvariety check; *Differences slightly significant; ** Differences significant;

NS= Non-significant differences; Diseases score: 1 – 3 = Resistant; 4 – 6 Intermidiate; 7 – 9 = Susceptible reaction

2.3 Participatory selection of drought and heat tolerant climbing bean varieties

ISAR introduced 56 climbing bean lines that were bred for adaptation in mid altitude conditions climbers (MAC) from at CIAT They were evaluated in observation nursery, preliminary and intermediate yield trials at Rubona (1650 masl), Karama (1200 masl) and Nyagatare (1400 masl) research stations over 6 seasons. During 2007 and 2008 the 5 most promising lines and local farmers check (local mixture) were planted in replicated multilocation yield trials and were evaluated by farmer participatory method for yield, tolerance to biotic, abiotic stresses and other agronomic and socio-economic attributes across 12 different locations in Nyagatare and Bugesera districts of the eastern region. The recorded data, ANOVA (by Genestat) and farmers' perceptions of the different entries revealed significant differences in yield, plant vigour, duration to flower and to mature and in tolerance to the most prevalent diseases: common bacterial blight, bean common mosaic virus, angular leaf spot and leaf rust (rated using a CIAT scale of 1 – 9) (Table 2). The study succeeded in identifying 3 new climbing bean varieties: **MAC 49, MAC 9** and **MAC 44** that had yield potential of 3.3 to 3.6 t ha^{-1}, about 2 to 2.5 times more than the popular mixed bush variety (local mixture) check's. They were relatively early maturing (85 – 87 days), compared to climbers adapted to higher altitude 1600 – 2300 masl that mature in 100 – 120 days. Besides being resistant or tolerant to prevalent major diseases (severity score 1- 5), they were appreciated by farmers due to their medium or large red mottled (Calima) seed type that are associated with fast cooking, appealing broth colour, taste and premiums on the market. They were released as **Gitego 1, Gitego 2 and Gitego 3** respectively (in January 2010), for farmers in the drought-prone environments of eastern Rwanda. **"Gitego"** is a local word, meaning **"a score"**.

Table 2. Yield performance across 15 locations, ANOVA and Duncun's Multiple Range Test (DMRT) and farmers ranking of newly released drought tolerant climbing bean varieties in Rwanda

Variety	Mean yield (kg/ha)	DMRT*	% yield over check	Farmers' ranking
MAC 49	**3600**	**A**	**181**	**3**
MAC 09	**3400**	**AB**	**198**	**2**
MAC 42	3300	AB	184	4
MAC 44	**3000**	**AB**	**162**	**1**
MAC 38	2600	BC	141	5
Local mixture (Check)	1800	C	100	6
Significance by check ($P_{0.05}$)		***		
C.V. (%)		*12.9*		

*Yields values accompanied by the same letter(s) are not statistically significantly different

4. Seed multiplication and dissemination

To enhance the availability of seed and adoption of new improved varieties by farmers in Nyagatare, Gatsibo and Kabarore Districts in Umutara, and in Bugesera semi-arid zones, more than 6 tons of breeder and pre-basic seed of new drought tolerant bush, semi-climbing and climbing bean varieties (**SER 16, SER 30, MAC 9, Mac 44 and MAC 49**); and old varieties **RWR 1668, RWR 2245, RWR 1180, RWK 10** that are adapted to the region for their early maturity, desirable seed types and high yields were multiplied in the 2 stations of Nyagatare and Karama in 2009. They were distributed to the new seed company (RWASECO), private farmers, partner NGOs and farmers' cooperatives. The seed has catalyzed the secondary seed multiplication, the dissemination and adoption by the farmers of the new and old ISAR varieties.

5. Training of farmers

In order to transfer skills and capacity for modern technologies of beans production, 150 farmers from Nyagatare, Gatsibo and Kabarole of Umutara project area were trained on production of good quality seed and on how to identify and manage key bean diseases and soil related constraints. They were

also trained on the importance of planting improved seed and varieties, soil fertility and good husbandry practices of beans in the field and post-harvest.

6. Participatory variety selection (PVS)

There was a training workshop on PVS that involved 31 participants that represented many organizations (NGOs, Farmers' Cooperatives, Government extension, ISAR scientists and technical staff) based in the project area of operation of Umutara and Bugesera. The participants were able to conduct a successful farmer participatory variety selection exercise in the Bugesera zone of the project. The same group of participants has been regularly involved with subsequent PVS in the project sites of Umutara and Bugesera areas.

7. Farmers' preferences and selection criteria of drought resistant varieties

These have been diverse. The most frequently mentioned were:

1. Early uniform maturity to escape terminal drought
2. Green leaf canopy where others are wilty/dried as a token of water-use efficiency
3. Strong and firm stems that don't log easily and associated with deep root system
4. Strong and firm stems that can support heavy pod loads
5. Podding uniformly distributed from the base upwards (upper pods preferred under rainy conditions)

Other general (newly heard) selection criteria that were mentioned were:

1. Green pods that are easy to open (unshell) – some pods take time and energy to open
2. Can be cooked with one log of "fire wood" – fast cooking observed from seed-type
3. Small seed associated with high density (Mass per unit volume) that is good for pricing once sold by weighing balance, but at loss once sold by sacs
4. Conversely, large seeded fetch more prices by sacs and less by weighing
5. Some bean types are apparently "oily" and won't stick to local cookers after cooking and so easy to clean
6. Clear/shiny red seed coat associated with "blood"- meaning vitamins and so good nutritional quality
7. Less causes of flatulence associated with certain seed types (yellows, khakis, white or light coloured

8. Main lessons learned

1. Ranking of same set of varieties can change depending on time of evaluation; season and environment, and evaluators. This can be confusing if scientists can so much rely on farmers' selection criteria to release varieties.
2. Using same group of farmers more than once/twice can make the PVS a routine, with tendency to have same or similar responses during discussions
3. However, "secret balloting" / placement of ribbons in envelops; different colors of ribbons used in selection for men and women, questioning skills during discussions were key components of the PVS exercises.

9. Planning workshops with partners

These have been organized, at least once at the beginning of a season. These were initiated by ISAR or the other partners. Joint action plans were usually made and monitored. The most recent was a regional workshop organized by PABRA in Bujumbura, Burundi (for DR Congo, Burundi, Kenya, Uganda and Rwanda), where all major national partners in research and in seed multiplication participated, shared experiences and developed a seed multiplication and variety dissemination plan of action.

10. Publications

1. There is a draft article for publication in a referred journal on *"Farmer Participatory Selection of Climbing Beans Adapted to the Semi-Arid Zones in Rwanda"* by **Augustine Musoni et al.** This was published as an abstract in an International Conference by AGRA in Mali last year.

2. A student presented a thesis on *"Adaptability of Climbing and Bush bean Varieties in Semi-Arid Nyagatare District"* by **Twahirwa Emmanuel**, under the supervision of **Augustine Musoni**. The thesis was graded as a "Distinction".

DARS-Bean Program, Malawi
Contributors: W. I. H. Makumba and I. R. Fandika
Introduction: Common bean is a very important source of vegetable protein and income for farmers in Malawi. However, the production of beans is constrained by (1) low soil fertility that has been exacerbated by acid conditions of the soils and (2) recurrent droughts. Many farmers have lost their bean germplasm due to either midseason dry spells or droughts whereby their crops have dried completely requiring them to re-plant a new crop. The current varieties we have in Malawi are not tolerant to Al toxicity hence production of beans in these soils is very low. The first option to improve the bean yield under Al and drought stress conditions is breeding varieties that are tolerant to these stresses. DARS was subcontracted by CIAT to evaluate drought and aluminum toxicity tolerant bean lines developed by CIAT. The purpose of the project is to evaluate drought and Al tolerant bean lines that whould maintain high yields under water stress and acidic soil; and to increase benefits to resource-poor farmers from stress-adapted, improved common beans. The trials were conducted in strong acid soils and also subjected to water stress both on station and farmers' fields. Farmers participated in the evaluation of the beans and selected the most preferred lines.

In the first year we started with 80 bean lines that included stress adapted bean lines from Malawi and Colombia, F8 drought adapted sugar lines and F8 low P adapted sugar lines. The lines were evaluated for tolerance to (i) drought at Kasinthula and Bembeke Research Stations, in farmers, fields in Kasungu and (ii) Al toxicity at Bembeke research station. The beans were also planted at Chitedze in Lilongwe. At all the stations the trials were subjected to participatory variety selection (PVS) by farmers to get the farmers' views and preferences on the introduced lines.

Tolerance to drought: The bean lines were grown under irrigation so that we could mimic the drought situation to stress the beans, hence the following two main treatments were derived:

(1) Non drought stressed: where water was applied at regular intervals from the date of planting through to crop maturity maintaining water moisture above 60% of the soil's water holding capacity;

(2) Drought stressed: in this treatment the crop was irrigated only during the early growing stages and irrigation was discontinued at 50% flowering stage, thereafter the crop grew on residual moisture. During the dry season experimentation we did not receive rain.

Drought Susceptibility Index (DSI) was calculated as DSI = 1- Y_s/Y_{ns} (Fisher and Maurer, 1978) where Y_s is the yield of stressed beans and Y_{ns} is the yield of non stressed beans.
Tolerance to Al toxicity: Another set of bean lines that included: New Bilfa, Ex- Malawi, Ex-Colombia, F8 Sugars and F8 Low P sugars, were further evaluated for tolerance to Al toxicity. The beans were evaluated at Bembeke Sub-research Station, in acid soils with pH ranging from 4.4 in the top 0-10 cm to 6.6 in the 40-60 cm soil layer, the concentration of Al in the soil ranged between 0.5 and 6.0 ppm.
Plot layout and replication: The beans were planted on ridges spaced at 60 cm apart, one bean seed was planted per planting hill at 10 cm apart. There were four ridges per each plot of 5 m long. The treatments were replicated four times. The treatments were arranged in a randomized complete block design with each replicate at each site treated as a block. Data was collected from the two central ridges.
Participatory Variety Selection: In the final assessment the farmers were asked to identify only the best variety. The participating farmers were grouped into three, one group consisting of male farmers only, second group comprising of female farmers only and the third group was a mixture of male and female farmers preferably having the same ratio. The farmers were given ribbons with which they used to tag their preferred bean line. Two sets of different colored ribbons were used to distinguish the gender of the selecting farmers. Female farmers were given a different color from the male farmers. The farmers were briefed on how to mark their preferred lines and before starting farmers were given a chance to go through the plots to preview the lines and compare their performances before making

decision on the best variety. A few pods were harvested from each treatment, opened and the beans were displayed on the plot for the farmers to see the seed color and size.

The farmers selected the lines basing on the following properties: number and sizes of pods per plant, stand count, time to maturity, tolerance to disease and pest pressure, growth vigor, marketability. The picture (Photo 1) below shows how farmers made their selection in a PVS.

Photo 1. Farmers conducting PVS in the Al tolerant bean trial at Bembeke Agricultural Research Station

Field Days: At each site a local field day was conducted involving farmers from the surrounding villages and sellers. Table 1 presents the statistics of the attendance to the field days.

Table 1. Stakeholders participating in the local field days held in selected sites

Site	Group of participants	No. of Men	No. of Women	Total
Bembeke	Farmers	11	21	32
	Sellers	9	7	16
NRC-LL	Farmers	19	26	45
	Consumers	9	17	26
Chinsewu	Farmers	13	15	28
	Sellers	3	11	14
Total Participants		**64**	**97**	**157**

Capacity building of national research scientists: The project sponsored one MSc. student, Mr. Bello Shano, who was registered with Bunda College of Agriculture, the University of Malawi. The student has finished the course work and research, has produced the first draft of his MSc thesis. He superimposed his studies in the trial established at Bembeke Agricultural Research Station. He studied the processes of Al, effects of Al on plants' root development and interaction of drought and Al toxicity.

The MSc student has finished collecting data for his thesis, data has been analyzed and first draft of the thesis has been submitted to his supervisors.

Soil properties at Bembeke Research Station and surrounding farmer fields: The soil reaction at Bembeke site was acidic to strongly acidic from 0 to 105 cm soil profile (Table 2). Available P is very low hence limiting to crop production, P concentration decreased with increasing depth. Soil K concentration is medium in the top soil in all the sites except for Kapenuka village. At the station K concentration increased with increasing soil depth from the top 0 to 105 cm depth. Soil organic matter levels were medium at the Station. Aluminum concentration was high in the top soil 0-15 cm at all the sites (Table 3).

Table 2. Soil properties of the farmers' fields at Bembeke in Dedza.

Site	Depth (cm)	Soil Texture (%)		pH	OM g kg^{-1}	N mg g^{-1}	P mg kg^{-1}	K cmol/kg
		Clay	Silt					
Bembeke	0-15	50	10	3.6	24	1.19	21	0.30
Research	15-30	52	10	3.8	34	1.72	12	0.31
Station	30-45	48	16	4.4	33	1.64	7.5	0.24
	45-60	46	12	4.5	31	1.56	5.6	0.58
	60-75	56	10	4.1	21	1.03	6.7	0.91
	75-90	58	10	4.1	16	0.78	5.6	0.86
	90-105	64	6	4.2	8	0.41	5.4	0.86
Farmers' fields around Bembeke Station								
Ngonoonda	0-15	26	14	4.7	13	0.66	27.1	0.44
Kapenuka	0-15	34	16	4.5	20	0.98	11.0	0.13
Kuthindi	0-15	42	16	4.5	10	0.49	8.5	0.27
Simuka	0-15	40	14	5.0	30	1.48	19.2	0.40

Table 3. Soil Al concentration in the 0-15 and 15-30 cm soil layers at Bembeke Agricultural Research Sub-station, Bokosi and Chinsewu village.

Site	Soil layer	Al (mmol/kg)
Bembeke Station	0-15	31
	15-30	15
Bokosi village*	0-15	14
	15-30	6
Chinsewu village*	0-15	26
	15-30	25

*At Bokosi and Chinsewu, Soil Al^{3+} concentration was only determined for the soils with very low pH values.

Results on seed yield for drought tolerant bean evaluation: The first two highest yielding bean lines under non-drought stressed conditions coincidentally were again highest yielding lines under stressed conditions; however, the percent yield losses were not the lowest. Table 4 gives the top 20 highest yielding lines and the farmers' preferences. The results show that farmers' preferences did not directly match with the bean yield since the farmers were also looking at seed size and the seed color. Therefore, the highest yielding lines did not have the traits preferred by the farmers. The farmers'

preference ranked VTTT 925/9-1-2 as number 1 and BF13572-5 as second and yet BF 13572-5 ranks first by yield.

BF 13572-5 yielded highest in both Stressed and non stressed conditions. The yield loss after drought stressing ranged between 1 and 43%. The bean lines with low yielding potential had the lowest yield loss percentage when subjected to drought stress (Table 5).

Table 4. The first 20 highest yielding drought tolerant bean lines versus farmer preference in 2009.

ID of Cross	Origin of materials	Bean Yield (kg/ha)		Yield loss (%)	Farmers' preference
		Non Stressed	Stressed		
BF13572-5	NEW BILFA	2060	1559	24.32	12
SER 83	EX-COLUMBIA	1702	1498	11.99	4
CAL143/SUGAR131	F8 FARMERS SELECTION	1682	1074	36.15	9
VTTT925/9-1-2	NEW BILFA	1676	1439	14.14	22
SER 45	EX-COLUMBIA	1665	1071	35.68	0
SER 85	EX-COLUMBIA	1654	1152	30.35	2
MR13456-12-2	NEW BILFA	1611	1147	28.80	0
BF13607-8	NEW BILFA	1608	915	43.10	1
PC652-553/VAX5	F8 FARMERS SELECTION	1554	1118	28.06	3
SER 55	EX-COLUMBIA	1543	1493	3.24	0
SER 79	EX-COLUMBIA	1541	1223	20.64	1
TIOCANERA75	EX-MALAWI	1518	1183	22.07	0
BF13607-12	NEW BILFA	1503	1219	18.90	3
SER 43	EX-COLUMBIA	1489	1393	6.45	1
SER 81	EX-COLUMBIA	1483	1414	4.65	3
SER 80	EX-COLUMBIA	1452	1255	13.57	1
SX14337-6	F8 SUGARS DT. LINES	1439	908	36.90	2
SER 53	EX-COLUMBIA	1431	1413	1.26	0
SXB 405	EX-COLUMBIA	1426	1183	17.04	4
SX14337-8	F8 SUGARS DT. LINES	1419	833	41.30	9

SER 124 bean line out yielded the control, CAL 143, by 52.9% followed by SER 83 with 47.2% and SER 43 and BF 136007-12 (33.4%) under drought stressed conditions. Under non drought stressed conditions the best five high yielding bean lines include: XB 405, BF 13607-7, SXB 413, SER 83 and BF 13572-5. The DSI for all the beans were below 0.5 showing that the introduced lines did not succumb to drought stress (Table 6).

The superior lines selected from drought stress trial were evaluated for their response and potential yield in different areas under rain fed (Table 7). SER 83 gave the highest average yield (2129 kg/ha) across the sites followed by SER 45 with an average yield of 1982 kg/ha. Almost all the introduced bean lines yielded higher than the check bean variety, Cal 143 except for PC652-553/VAX5, BF 13607-12, SER 72, SER 78, SXB 413, VTTT924/1-9-8-1 and VTT 925/9-1-2.

Table 5. Bean yield under drought stressed and non drought stressed conditions, Kasinthula Agricultural Research Station.

Bean Line	Grain weight, kg/ha		DSI[1]	% Yield increase over control	
	Stressed	Non Stressed		Stressed	Non Stressed
BF 13572-5	2131	3067	0.31	14.27	42.27
BF 13607-12	2488	2704	0.08	33.40	25.44
BF 13607-9	2011	3478	0.42	7.84	61.37
CAL 143	1865	2156	0.13	0.00	0.00
MR 13456	1795	2701	0.34	-3.78	25.31
MR 14215-9	2174	2260	0.04	16.57	4.84
PC652-553/VAX5	1169	2163	0.46	-37.31	0.32
SER 124	2852	2902	0.02	52.91	34.62
SER 43	2488	2665	0.07	33.38	23.65
SER 45	1932	2737	0.29	3.59	26.98
SER 53	1784	2682	0.33	-4.35	24.40
SER 55	2160	2537	0.15	15.81	17.69
SER 72	2087	2628	0.21	11.87	21.90
SER 75	2006	2464	0.19	7.57	14.29
SER 78	2400	2919	0.18	28.66	35.40
SER 79	1963	2156	0.09	5.26	0.04
SER 80	2295	2320	0.01	23.04	7.63
SER 81	1694	2317	0.27	-9.19	7.51
SER 83	2745	3228	0.15	47.17	49.77
SER 85	2031	2813	0.28	8.88	30.51
SXB 405	2267	3530	0.36	21.56	63.76
SXB 413	1858	3289	0.44	-0.39	52.60
TIOCANERA 75	1707	2515	0.32	-8.48	16.67
VTTT924/19-8-1	1946	2171	0.10	4.35	0.74
VTTT925/9-1-2	1926	3052	0.37	3.25	41.57
Mean	**2071**	**2698**	**0.22**	11.04	25.17
$LSD_{0.05}$ Beanline		NS			
$LSD_{0.05}$ Stress		186.1			
$LSD_{0.05}$ Beanline x Stress		821.7			
CV%		25.5			

[1]DSI = Drought Susceptibility Index.

Table 6. The top five varieties for the three years, from 2007/08 to 2009/10, under drought stressed conditions at Kasinthula.

Rank	2007/8	2008/9	2009/10
1	SER 85	BF13572-5	SER 124
2	BF 13607-6	SER 83	SER 83
3	PC 652-553 VAX5	VTTT925/9-1-2	BF 13607-12
4	TiOCanera 75	SER 45	SER 43
5	SER 45	SER 85	SER 78

Table 7. Bean yield at various locations in Malawi grown under rain fed conditions in 2010.

Bean line	NRC-LL	Chitedze	Chinsewu	Bembeke	Bokosi	Mean
BF13572-5	1388	2622	1992	290	1499	**1558**
BF13607-12	1818	1510	1422	359	1368	**1295**
BF13607-9	1671	2361	1623	482	1812	**1590**
CAL 143	2454	2535	705	545	853	**1418**
MR13456-12-3	1312	3275	2469	543	1513	**1822**
MR14215-9	2014	1979	1873	315	1317	**1500**
PC652-553/VAX5	1014	1771	838	525	813	**992**
SER124	1414	2049	1979	316	1429	**1437**
SER43	1358	2500	2211	434	1863	**1673**
SER45	2476	2685	2507	365	1878	**1982**
SER53	1791	2326	2855	611	1463	**1809**
SER55	1378	1958	2190	763	1400	**1538**
SER72	1651	1858	1010	480	1507	**1301**
SER75	1702	2431	1618	689	979	**1484**
SER78	1193	2778	741	440	1690	**1368**
SER79	1657	3351	1028	535	1634	**1641**
SER80	1423	2378	1454	474	1564	**1459**
SER81	1620	2691	1655	373	1255	**1519**
SER83	2649	3299	2498	702	1497	**2129**
SER85	1120	3194	2093	397	1636	**1688**
SUG 131	1993	1719	672	315	1114	**1163**
SXB405	1220	3194	1872	614	1608	**1702**
SXB413	1591	2448	1450	353	1097	**1388**
TIO CANERA75	1658	2865	1598	553	1233	**1581**
VTTT924/1-9-8-1	1792	1580	612	602	1278	**1173**
VTTT925/9-1-2	2330	2135	320	600	959	**1269**
Mean	**1680**	**2442**	**1588**	**487**	**1395**	**1518**
S.E.D. $_{0.05}$	425.7	469.3	552.5	NS	163.6	
CV%	35.5	41.8	32.2	43.3	40.1	

Evaluation of Bean lines tolerant to Al, Bembeke on Station trials: The bean yields at Bembeke are the lowest compared to all the other sites where the same lines were evaluated for drought as shown in Table 7. However, out of the 26 bean lines tested in the five sites only SER 55, SER 83 and SER 72 showed to be generally higher yielding than the others. In acid soils the check variety out performed over 50% of the newly introduced lines. These lines easily succumb to Al toxicity.

Relationship between Al and bean roots distribution: The root length of beans decreased with increasing Al^{3+} concentration in the soil thus increasing soil acidity reduced the root length of the beans (Figure 1). High Al^{3+} concentration in the soil impeded the development of the roots this in turn affected the aerial biomass production as the crop was able to explore larger volume of soil to absorb more nutrients. The two pictures in Photo 1 show bean root distribution in limed and non limed soils. The picture at the left is non limed soils, the roots grow laterally along the ridge whereas on the right the roots are growing more vertically. The bean crop at the right has many roots in the top 0-20 cm and also the roots anchor deep into the sub-soil whereas the crop affected by Al toxicity has few roots developed within 0-10 cm and do not extend beyond 20 cm soil depth.

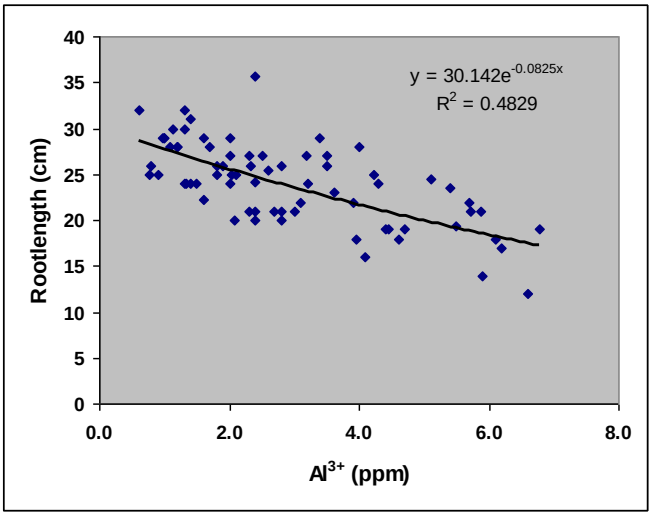

Figure 1. The effect of exchangeable Al on bean root development (root length)

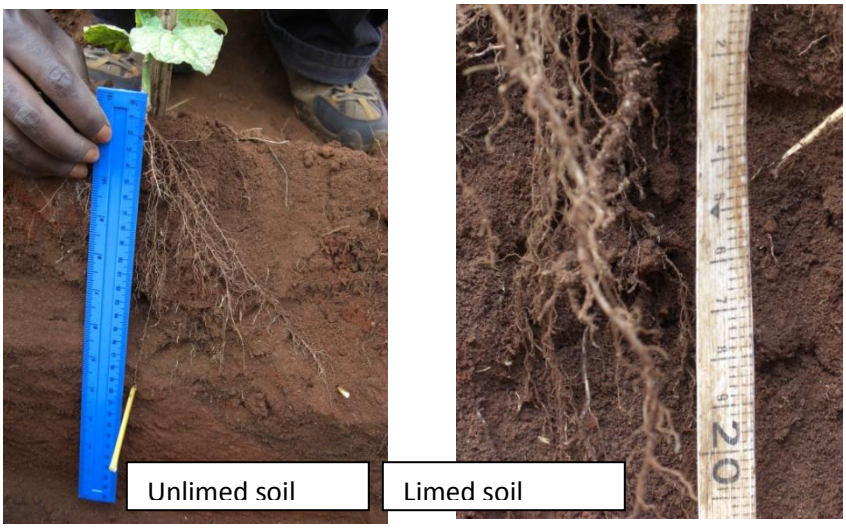

Photo 2. Bean root distribution in acid non limed and limed soils at Bembeke Agricultural Research Station

Farmer preferences: The farmers in all the sites were invited to evaluate the bean lines, using a procedure of PVS. In addition to the growth response of different lines that were tested we were able to get the farmers' preferences. Table 8 presents the best five bean lines selected from each site. Although bean yields were different from one site to another the farmers' choices seemed to be common across the sites as most farmers were looking for the following traits: Seed size, colour, yield potential, ability to cook fast, tolerance to diseases and drought stress. Although high yielding was one of factors used in selecting the beans it was not a priority as some high yielding varieties but with dull colours were rejected.

Table 8. Farmer preferred bean lines.

Site	Bean lines
Kasinthula	VTTT925/9-1-2, BF 13572-5, SER 124, SER 78, SER 83
Bembeke	VTTT 925/9-1-2, MR 14215-9, SER 83, SER 55, SER 53
NRC-Lilongwe	VTTT 925/9-1-2, SER 83, SER 45, MR 14215-9, BF13607-12
Bokosi	VTTT 925/9-1-2, BF 13607-2, SER 83, SER 78, SER 45
Chinsewu	VTTT 925/9-1-2, MR 14215-9, SER 78, VTTT924/1-9-8-1, SXB 405

Conclusions

The results have shown that farmers prefer VTTT925/9-1-2, BF 13572-5 and SER 83 but also yielded higher than the control CAL 143 under drought stress.

Under Al toxicity the following three lines gave high yields showing that they were tolerant to Al toxicity: SER 55, SER 83 and SER 75

Recommendation

Two bean genotypes, VTTT925/9-1-2 and BF 13572-5, have been selected by farmers based on good table and market traits and these two genotypes also have high yielding potential under stress conditions hence are recommended for release so that the farmers can start growing.

ISAR-Forages,Rwanda

Participatory evaluation of Brachiaria grass options in Rwanda

Contributors: Mupenzi Mutimura, Myambi Celestin, Lussa André, and Cyprian Ebong

Summary: Shortage of animal feed is a major constraint for livestock development in Rwanda. Growing grasses of non-improved forage species and lack of appropriate technologies to manage natural resources contribute to the problem of fodder shortage for smallholder farmers in Rwanda. The deficiency in both quality and quantity of feeds has arisen from shrinking pasturelands, poor quality of both natural and commercialised feeds, water shortage and limited use of crop residues and agro-industrial by-products. Agriculture in Rwanda, including Bugesera and Nyamagabe districts, is based on smallholder mixed crop-livestock farming systems with land holdings of $\leq$ 0.5 hectare for the majority of farmers. To address this issue, the Rwanda Agricultural Research Institute (ISAR) in collaboration with the International Centre for Tropical Agriculture (CIAT) evaluated *Brachiaria* grass options with farmers in contrasting drought (Bugesera district) and acidic soil (Nyamagabe district) environments in Rwanda.

This work is part of BMZ-GTZ, Germany funded project "Fighting drought and aluminium toxicity: Integrating functional genomics, phenotypic screening and participatory evaluation with farmers to develop stress-resistant common bean and *Brachiaria* for the tropics". This report summarizes the results of research carried out from March 2006 to March 2010. The objectives were: to (i) identify feed resource-use patterns under drought, acidity and aluminium toxicity stress conditions, (ii) analyse the role of gender and wealth categories in livestock activities in the target areas, (iii) assess the production and forage quality of improved and indigenous *Brachiaria* grasses and buffelgrass (*Cenchrus ciliaris*) (iv) assess the farmers' perception of the new varieties and hybrids of *Brachiaria* grass, (v) determine farmers' criteria for selecting fodder species, and (vi) assess the level of early adoption of new *Brachiaria* by farmers in selected sites.

Focus group discussions were held by 20 farmer representatives from each district. Farmers were divided into two groups of males and females, and each group drew up livestock activities related to gender. In each district, 20 farmers identified criteria to rank the known feed resources. Individual farmers gave scores to each feed resource according to farmers' criteria, and the scores were considered as quantitative indicators of farmers' preferences.

In both districts, livestock activities were shared between genders, but certain activities (e.g., milking cows, animal shed construction) were restricted to males due to cultural beliefs. In both districts, the study showed that both the number and type of cattle were important in defining wealth ranking and status within the community. Assessing the different forages in the low rainfall district (Bugesera), *Pennisetum purpureum* (Napier grass) was given the highest score, in view of its availability all year round, followed by indigenous grass. In the acidic soil area district (Nyamagabe), Napier grass and *Commelina benghalensis* were scored high, the indigenous grass *Panicum maximum*, maize stovers (crop residue), and the indigenous tree *Albizia amygdalina* were also considered important feeds. The preference ranking confirmed that Napier grass is currently the major feed source used throughout the two districts. The results for availability, quality and quantity of feeds showed a shortage of livestock feed resources in both districts indicating a need for suitable forage species adapted to low rainfall and acidic soils.

Three varieties and five hybrids of *Brachiaria* grass obtained from CIAT and two local grasses (control) were used for on-farm participatory trials without fertilizer application. Twelve farmers were selected in each study area; ten grasses were established in 2 m x 3 m plots. Herbage was harvested six times during the year at two-monthly intervals. For each cut, dry matter (DM) was measured. Crude protein (CP), calcium (Ca) and phosphorus (P) contents were assessed once in the wet season and once in the dry season. In the low rainfall area, *Brachiaria brizantha* cv. Toledo and *Brachiaria decumbens* (local) had the highest DM yields (5.71 and 5.61 t ha^{-1}, respectively), while DM for the other grasses ranged from 1.2 to 5.13 t ha^{-1}. In the acidic soil area, *Brachiaria* hybrid BR02/1485 had highest DM yields with 5.95 t ha^{-1}, while other grasses yielded between 1 and 4.47 t ha^{-1}. The highest quality grasses were *Brachiaria* hybrid BR02/1485 with 12.2% CP in the low rainfall area and cv. Mulato II with 11.6% CP in the acidic soil area.

In the low rainfall area, *Brachiaria* hybrid cv. Mulato obtained a high mean Ca value of 2.15%, while in the acidic soil area, cv. Marandu obtained a high Ca value of 2.41% during the wet and dry seasons. Cv. Toledo had a high P concentration (0.28%) compared to the other grasses (0.07–0.11%) in the low rainfall area. In the acidic soil area, *Brachiaria* hybrid BR02/1485 had a higher P concentration of 0.53% compared to other grasses in which P varied between 0.16 and 0.47%. Local control grasses had lower nutrient contents than improved *Brachiaria* grasses in both the low rainfall and acidic soil areas. Although cv. Mulato II was not the most productive grass, it was selected by farmers as a preferred cultivar at both sites because of its adaptability to low rainfall and acidic soil stress, and its production of green forage year round without any fertilizer input.

In both districts, farmers have adopted the new forage alternatives by increasing the sizes of their plots and forming cooperatives to produce planting materials of the best bet *Brachiaria* grass options. The five *Brachiaria* grass options that were used for multiplication across the two contrasting districts were *Brachiaria* hybrid cv. Mulato II, *Brachiaria brizantha* cv. Toledo, *Brachiaria brizantha* cv. Marandu, *Brachiaria* hybrid BRO2/1485 and *Brachiaria decumbens* cv. Basilisk. These grasses have been selected for multiplication by farmers under both stress conditions because of their adaptability to drought and acidic soil, and producing high forage biomass throughout the year.

Training on forages, feeding and participatory research approaches performed at different levels (farmers, technicians and extension workers, academic) will assist knowledge transfer within the country.

1. Introduction

Natural pastures are important feed for livestock in Rwanda. However, they mature rapidly and become very fibrous and, thus, loose quality. Grazing lands are also sharply shrinking because land allocated to food crop cultivation is increasing to satisfy the needs of the increasing human population. Although crop residues are valuable feed resources, their nutritional values are often too low to sustain ruminant production.

The predominant type of agriculture in the districts of Rwanda, including Bugesera and Nyamagabe districts, is integrated crop and livestock farming on small land holdings ($\leq$ 0.5 ha) for the majority of

farmers. The system benefits from complementarities between crops and livestock through provision of residues for animal feed, and manure for crop production.

Agriculture in Nyamagabe is constrained by low fertility, acidity, and aluminium toxicity of the soils. In Bugesera district seasonal drought is the main constraint. Adapted crop and forage germplasm is therefore needed to enhance the productivity and resilience of these crop-livestock systems. By integrating high-yielding forage species into existing cropping systems, positive effects on crop productivity and feed output are anticipated, reducing competition for land

Studies carried out in the southern province of Rwanda (now Nyamagabe district) have reported crop failures in bush beans due to soil acidity and aluminium toxicity. To address this issue, the Rwanda Agricultural Research Institute (ISAR) and the International Center for Tropical Agriculture (CIAT) carried out research trials and were able to recommend climbing beans, which could resist this stress. However, less attention has been given to the poor quantity and quality of forage for livestock. Therefore, a collaborative project between CIAT and NARS, including ISAR entitled "Fighting drought and aluminium toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics"* was carried out, with funding by BMZ-GTZ.

The project goal was to contribute to food security and sustainability of crop-livestock systems in tropical areas prone to drought stress and aluminium (Al) toxicity. The project purposes were: 1) to discover drought and Al resistance/tolerant candidate genes that are involved in maintaining root elongation under stress; 2) to develop phenotypic and molecular tools to facilitate marker-assisted selection (MAS) in common (*Phaseolus*) bean and *Brachiaria*; and 3) to increase benefits to resource-poor farmers from stress-adapted, improved common bean and *Brachiaria*.

In this project, ISAR contributed to output 1: *Rural benefits enhanced in the target areas of tropical Africa and Central America by involving women and small-scale farmers as decision makers and co-researchers in the common bean and Brachiaria product development process.*

The activities were:

(i) Exploratory diagnosis/assessment of current forage use patterns.

(ii) Actor linkage mapping and capacity enhancement of existing innovation systems for improved forage productivity.

(iii) Pilot testing of new accessions of *Brachiaria* in sites with high potential for improving milk and meat production.

(iv) Community-based evaluation of opportunities for adoption of new beans and forage varieties using farmers' decision-making criteria and trade-offs among traits.

(v) On-farm testing and evaluation - under both researcher and farmer design at different farm niches, and with various management practices. For *Brachiaria,* specific end-uses were monitored.

(vi) Adoption studies of farmer integration of new products into existing farming systems, after several seasons.

(vii) Pre-release multiplication and dissemination of farmer-selected varieties via multiple channels (local and regional, public and private). This activity premised on the fact that, while bean seed channels are well known, some initial assessment for forage seed multiplication may be required.

(viii) Human capacity building: MSc training of ISAR forage scientist using *Brachiaria* on-farm evaluation activity formed a part of MSc studies of Mupenzi Mutimura (from ISAR- Rwanda) who benefited from a scholarship in this project to contribute to its output 1. Results reported here, are from the summary of Mupenzi Mutimura's MSc thesis, of which a copy will be sent to CIAT.

2 Participatory Rural Appraisal (PRA) in Rwanda

2.1 Selection of pilot sites in Rwanda

Two sites with contrasting farming systems were selected for the participatory evaluation of forages. The Nyamagabe district was selected for aluminium toxicity of soils, medium - high altitude (1800 m), and intensive integrated crop-livestock farming under cut-and-carry forage systems. Bugesera district was selected for drought stress, low altitude (1400 m) and extensive farming system.

2.2 Farmer selection

The farmer selection process was guided by local extension personnel to identify farmers with interest and need in new forage production options for an intensive management system in the target districts (Bugesera and the Nyamagabe). A rapid appraisal of production systems showed that the most

common forages encountered were Napier grass (*Pennisetum purpureum)* and the fodder tree *Calliandra* spp.
Most farmers possess 1 to 5 cows (Plate 1 and 2), supported by government policy and different NGOs providing improved livestock and artificial insemination to improve the genetic base of the animals.

Plate 1. Farmer Joseph in his Napier grass plot and his cows

2.3 Training field staff and scientists in participatory research approaches for forages
A ten-day training workshop was done in October 2006 at ISAR RUBONA. The objective of the workshop was to equip researchers and field workers with approaches for participatory forage evaluation and forage agronomy. During the workshop, participants from wards and NGOs of Bugesera and Nyamagabe shared knowledge of forage agronomy with smallholder farmers.
After site selection and training, the field staff and scientists conducted a participatory rural appraisal (PRA). The tools used included gender analysis, wealth ranking and feed calendar development. Preparatory meetings were held with the livestock owners who volunteered to participate in the study at Nyamata and Gasaka sectors in Bugesera and Nyamagabe districts, respectively. The aim of these meetings was to develop consensus with farmers on the objectives, processes, expected outputs, as well as the use of participatory rural appraisal (PRA) tools of the study.
Prior to the PRA exercise, arrangements were made with agriculture and livestock service providers in each district to visit selected farmers. The service providers were the representatives of organizations that supplied improved dairy cattle to farmers (Ministry of Agriculture and Animal Resources (MINAGRI), Heifer Program International (HPI) and Send a Cow Rwanda (SCR) at sector level. The meetings were held during the dry season of August 2007 when farmers were more available due to reduced field activities than in the cropping season. The specific objective of the household level visits was to determine the distribution of wealth within the community based on assets they owned and income generated. The results were used to determine the link between livestock ownership (critical herd sizes) and wealth; and between wealth standard and social status; as well as farmers' motivation to invest in forages for dairy farming. This was contrasted with the assessment of current feed options in terms of proportion and availability across seasons of the year.

2.4 Wealth ranking
Farmers first developed categories of wealth for the community. Thereafter they discussed, agreed and allocated individual farmers from the list of livestock owners.
In both districts of Bugesera and Nyamagabe, farmers defined five wealth categories among livestock keepers: the 'very rich', the 'rich', the 'moderately poor', the 'poor' and 'very poor'.
In the Bugesera district, the number of livestock owned by farmers was one of the major criteria used by farmers in allocating community members to the different social groups. The majority of people (75%) in the selected cells of the Bugesera district were in the category of 'moderately poor'. Many of them owned land of less than 0.5 ha and reared one indigenous cattle. Even though some farmers had an exotic dairy cow provided by the government of Rwanda or NGO, ownership was used as selection criteria for differentiation.
The categories of the 'very rich' and 'rich' were distinguished by the amount of money they possessed and daily cash income. However, the 'moderately poor' and 'poor' were differentiated by the availability of food. The last wealth category, the 'very poor' (UMUTINDI NYAKUJYA), did not own animals or land. They were often dependent on charity, and lacked esteem within the community.

In the Nyamagabe district, although results on wealth ranking also showed five categories of farmers, the characteristics identified by farmers within each category were different. The 'very rich' referred to cash income and other material possessions. The 'rich' had access to properties and food. The number and genotype of cows owned by farmers were among the major criteria for allocating households according to social welfare and status in the community in the district. The 'very rich' category owned exotic dairy cows, whereas the number of other breeds of cows designated the households into 'rich' or 'moderately poor' wealth categories. In the selected cells of the Nyamagabe district, 50% of the farmers were in the category of 'moderately poor' whereas 18.75% were in the category of 'rich'.

Wealth ranking in the Bugesera and Nyamagabe districts showed that even though farmers are located in different areas of Rwanda, similar criteria were used to categorize the community in terms of social livelihood. However, the weighting of these criteria within each category differed across communities. Some criteria were unique to specific communities. For example in the Nyamagabe district, farmers considered the number of coffee trees a peculiar characteristic of the 'rich' and the 'moderately poor'. In the Bugesera district, the 'very rich' and 'rich' were differentiated by the number of properties owned whereas in the Nyamagabe district the 'very rich' and 'the rich' were defined by access to cash.

2.5. Role of gender in livestock in selected sites

When considering the role of gender in livestock activities in the Bugesera and Nyamagabe districts, it was apparent that different gender categories corresponded to different activities. In the Bugesera district, apart from cattle, farmers owned small ruminants (i.e., goats) and small stock (rabbits and chickens). Animal husbandry and related activities varied by gender. Both women and men herded and participated in exchanging (donation and sales) cattle. Rearing of goats, rabbits and chickens were restricted to women, girls and boys. Planting forage, construction of animal sheds, seeking grazing land and animal disease treatment were men's activities, whereas milking cows was reserved for men and boy's activities.

The only activities in animal rearing, which were common to both male and female adults and children was fetching water for cattle and feeding cattle in the shed. Women and girls cleaned animal sheds. The role of men tended to be more physically demanding on labour than tasks that women and children did. However, group discussions revealed that, when women and children were household heads, they did all activities. Men did not consider small stock (chickens, goats, and rabbits) as valuable animals; therefore rearing these animals was the exclusive role of women and children, who spent much of their time at home. Women sold small stock after consultation with their husbands. Men were in charge of selling cows with the approval of their wives. Gender group discussions also revealed that, children had no rights to sell or donate animals unless they were orphans and, thus, heads of households.

In the Nyamagabe district, the gender analysis on animal rearing revealed dissimilar results to those of the Bugesera district. Cattle rearing was done by men, women and boys, whereas goat keeping was done by women, girls and boys. Rabbits and chickens were managed by both girls and boys, whereas pigs were only kept by women and boys. The activities of planting forage, selling and donation of animals were reserved for men and women. The construction of animal sheds, treating ticks on cattle, and milking cows were carried out by men and boys. Fetching water for cattle was the activity for women, girls and boys in the Nyamagabe district, whereas the activity common across gender and age groups was cleaning animal sheds and cattle feeding. The only activity reserved for men alone was the treatment of animal diseases.

In the contrasting districts, common and variable gender roles differentiated by location were identified. Fetching water for the animals was a common activity for men and children in the Bugesera district, whereas in the Nyamagabe district, men do not fetch water for animals. Cattle feeding was the only activity executed by men, women, girls and boys in both districts. This is a crucial activity as in both districts a zero-grazing system is practised. Participants mentioned that cattle feeding takes time for farmers to get enough feed and that is why all categories of age and gender were involved in this activity. However, treatment of animal diseases remained men's activity in both Bugesera and Nyamagabe districts. Although many activities in livestock rearing were shared between males and females, others were still related to only male or female. In the Bugesera district cleaning of animal sheds was reserved for only females (women and girls), whereas milking cows and animal shed construction were confined to males (both men and boys) in the Bugesera and Nyamagabe districts. In

households headed by either women and/or children, all the activities could be carried out by women except milking a cow, as Rwandan culture does not allow a woman to milk a cow. In this situation, she should look for assistance from a male in the neighbourhood. In both area districts, most livestock activities were shared between genders in the zero grazing system; but cultural beliefs and tradition, which considered cattle as a sacred animal, prevented women from milking a cow, but allowed them to handle milk.

2.6 Assessment of animal feed resources in selected sites

During workshops with farmers, the availability and utilization of feeds throughout the year was defined using feed calendars. Results on ranking of feed resources in Bugesera according to farmers' criteria showed that Napier grass was the most preferred feed (Figure 1), followed by *Tripsacum* sp, *Setaria* sp. and banana stem. The ranking confirms the perception that Napier grass is the major forage crop used throughout Rwanda. The criteria for farmers to choose Napier included its forage availability throughout the year, palatability, adaptation to low soil fertility and drought, stomach fill, easy handling for cutting, and good regrowth. Many farmers were not sure, which forage option may result in higher milk yields. Preferences of farmers for forages differed across seasons (Table 1). In the wet season, Napier, native grass, sweet potato and bean peelings were the feed resources mostly used, while in the dry season mostly banana stems, Napier and native grass were used.

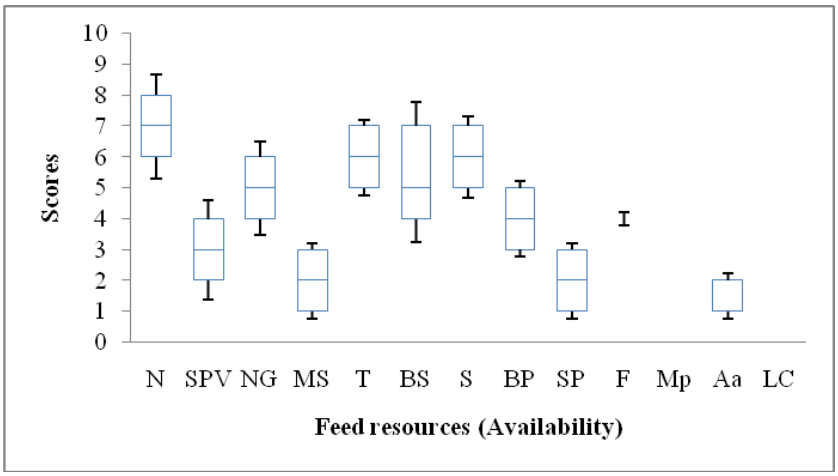

Figure 1: Boxplot of feed resources from farmers' preference ranking in the Bugesera district according to criteria of availability.

Key for feed resources in the Bugesera district:
N= Napier grass; SPV= Sweet potato vines; NG= Natural grass;
MS= Maize stover; T= *Tripsacum*; BS= Banana stems; S= *Setaria*;
BP= Bean peelings; SP= Sweet potatoes; F= *Ficus*; Mp= *Mucuna pruriens*;
Aa= *Albizia amygdalina;* LC= Leaves of cabbage.

Table 1. Typical Matrix Scoring of Feed resources by farmers in Bugesera.

Feeding system	Wet season (%)	Dry season (%)
Napier	100	70
Sweet potato vines	85	40
Native grass	100	60
Setaria	35	5
Tripsacum	30	10

Ficus	5	45
Albizia amygdalina	0	35
Maize stover	35	0
Bean peelings	70	0
Cabbage leaves	5	5
Sweet potatoes	20	15
Mucuna pruriens var. *utilis*	15	15
Banana stems	0	80

In Nyamagabe, *Commelina benghalensis* and Napier grass were the feed resource receiving the highest rank by the farmers (Figure 2), indicating the shortage of feed resources in the area. Napier, maize stover, *Panicum*, *Albizia* spp. and sweet potato were the other feed resources used by farmers. However, due to lower availability of feed, a wider range of feed resources with equal importance was employed (Table 2).

In contrast to Bugesera, in Nyamagabe bean peelings were used mostly in the dry season. In this drier environment of Bugesera, Napier and other grasses were mostly used in the wet season, while crop residues and fodder trees were used throughout the year.

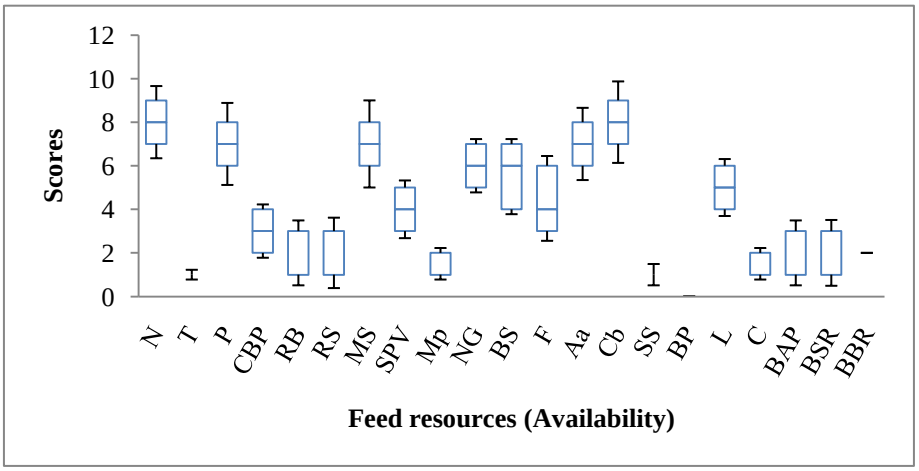

Figure 2. Boxplot of feed resources from farmers' preference ranking in the Nyamagabe district according to criteria of availability

Key for feed resources in the Nyamagabe district:
N= Napier grass; T= *Tripsacum*; P= *Panicum*; CBP= Cooked banana peelings;
RB= Rice bran; RS= Rice straw; MS= Maize stover; SPV= Sweet potato vines;
Mp= *Mucuna pruriens*; NG= Natural grass; BS= Banana stems; F= *Ficus* spp.;
Aa= *Albizia amygdalina;* Cb= *Commelina benghalensis;* SS= Suckers of Sorghum ;
BP= Bean peelings ; L= *Leucaena* spp.; C= *Calliandra* spp.; BAP= Banana peelings;
BSR= Beer sorghum residues ; BBR= Banana beer residues

Table 2. Typical Matrix Scoring of Feed Sources by Farmer in Nyamagabe.

Feeding system	Wet season (%)	Dry season (%)
Napier	50	25
Tripsacum	50	0
Panicum	50	15
Cooking banana peelings	50	50
Rice bran	15	15
Rice straw	15	15
Maize stover	15	25
Sweet potato vines	20	35
Mucuna pruriens	10	0
Natural grass	50	0
Banana stems	50	50
Ficus sp.	50	50
Albizia amygdalina	50	45
Commelina benghalensis	50	50
Sorghum suckers	0	25
Bean peelings	0	50
Leucaena sp	35	35
Calliandra sp	50	50
Banana peelings	25	25
Beer sorghum residues	20	20
Banana beer residues	15	15

3. On-farm evaluation of *Brachiaria* grass in selected sites

3.1. Materials and Methods

To implement some activities, a work plan was scheduled and consisted of two components:

1. Nursery establishment of multiplication of forage planting material
2. On-farm evaluation of *Brachiaria* grasses

3.1.1 Nursery of planting materials

For nursery establishment, the following materials were imported from Cali, Colombia to Rwanda: *Brachiaria decumbens* cv. Basilisk, *Brachiaria brizantha* cv. Toledo*, Brachiaria brizantha* cv. Marandu*, Brachiaria* hybrid cv. Mulato*, Brachiaria* hybrid cv. Mulato II*, Brachiaria* hybrid BR02/0465, *Brachiaria* hybrid BR02/1452*, Brachiaria* hybrid BR02/1485.

In addition, there was a need to multiply planting materials of local control treatments, which were *Brachiaria decumbens* (local) and buffelgrass .

The nursery was established at Karama in October 2006, with small quantities of seeds imported from CIAT, Colombia. Vegetative materials were ready to be planted in on-farm experiments for the rainy season starting in March 2007. The nursery contained 10 forage plots of 5 m x 5 m; 8 introduced *Brachiaria*, 2 controls including local *Brachiaria decumbens* and naturalised buffelgrass (Plate 1).

Plate 1. *Brachiaria* spp. in Nursery 3 weeks after sowing

Brachiaria brizantha (cv. Toledo) and *Brachiaria* hybrid (cv. Mulato II) showed good adaptation, growth and biomass (Plate 2 and 3) from germination to first cutting. *Brachiaria* hybrid (BR02/1452) had slow growth (Plate 4), likely due to excessive rainfall from sowing to germination and early growth.

Plate 2. *Brachiaria brizantha* (cv. Toledo)

Plate 3. *Brachiaria* hybrid (Mulato II)

Plate 4. *Brachiaria* hybrid (BR02/1452)

3.1.2 On-farm evaluation of *Brachiaria* grass options in Rwanda

An exploratory diagnosis of current forage use patterns and identification of pilot sites for *Brachiaria* was done. Pilot sites were identified for testing 10 forage grass options (including controls) with farmers, with high potential for improving milk and meat production. Grass-root level participatory research partners and farmer groups were identified to facilitate participatory diagnosis and planning. Actor linkage mapping was used to analyse existing innovation systems in forage production, and to increase the capacity to improve feed systems with *Brachiaria*.

In selected sites, the following activities were carried out: a) evaluate forage options via community-based forage trials to explore farmers' decision-making criteria and to understand trade-offs among traits; b) conduct on-farm testing and evaluation, addressing specific end-use and livestock types; c) conduct follow-up studies of farmer integration of new products into existing farming systems; and d) facilitate initial multiplication and dissemination of farmer-selected varieties via multiple channels (local and regional, public and private). The objectives of this study were (1) to determine the production and quality of improved *Brachiaria* grasses (varieties and hybrids) on-farm under low rainfall, acidity and aluminium toxicity stress conditions and (2) to determine the criteria of selection of new *Brachiaria* grass by farmers.

Eight improved *Brachiaria* grass options together with two local controls were established by vegetative material for on-farm participatory trials: 12 in Bugesera region (drought) and 12 in Nyamagabe region (Al-toxic acidic soil). Most *Brachiaria* plants established well across sites; however,

due to extended drought some replanting was needed. The field layout on-farm trial was as follows (Figure 1).

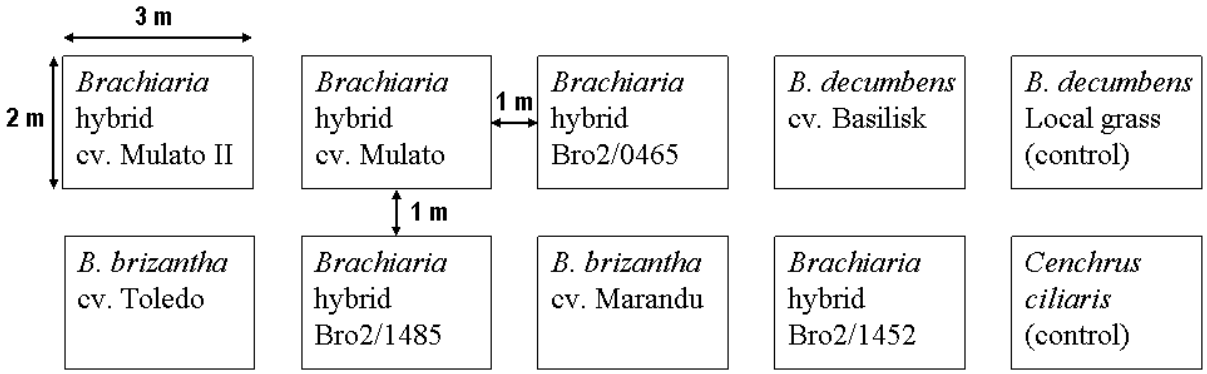

Figure 1. Field layout for on-farm *Brachiaria* establishment

Herbage was harvested in a 1 m^2 quadrat randomly placed within each 2 m x 3 m plot at each of the following harvest times:

- during peak of first rainy season (November 2007)
- during beginning of first dry season (January 2008)
- during the second rainy season (March 2008)
- during the end of second rainy season (May 2008)
- during the dry season (July 2008)
- during the rainy season (September 2008)

After collecting samples, the whole plot was cut to allow homogenous regrowth for the next cut. Samples were taken to the laboratory at Rubona Research Station of the Rwandan Agriculture Research Institute (ISAR) and at High Institute of Agriculture and Animal Husbandry of Busogo (ISAE Busogo) for nutritive value analysis. Samples taken from dried grasses were chemically analysed (once in the wet season and once in the dry season) for crude protein, phosphorus and calcium.

3.1.3 Results

3.1.3.1 Dry matter of grasses evaluated

Herbage productivity of *Brachiaria* cultivars and hybrids established on-farm by smallholder farmers of Bugesera (drought stress) and Nyamagabe (acid soil stress with Al toxicity) districts was assessed in both the rainy and dry seasons. Figure 2 shows the dry matter yield (kg ha^{-1}) of *Brachiaria* hybrids and accessions for the first rainy and dry seasons in Bugesera district. During the first rainy season, *Brachiaria* hybrids Mulato, Mulato II and *B. brizantha* cv. Marandu showed higher DM yield, whereas in the dry season *B. brizantha* cv. Toledo and *B. decumbens*/local presented the highest DM, followed by *B.* hybrid BR02/1485, Mulato, *B. decumbens* cv. Basilisk and cv. Mulato II. Over the two first seasons, *Cenchrus ciliaris* presented the lowest DM yield.

In Nyamagabe district, in both the rainy and dry seasons, *B.* hybrid BR02/1485 presented the highest DM yield (Figure 3), followed by Mulato, cv. Marandu and cv. Basilisk in the rainy season, and Mulato II, cv. Basilisk, cv. Marandu and *B. decumbens*/local in the dry season.

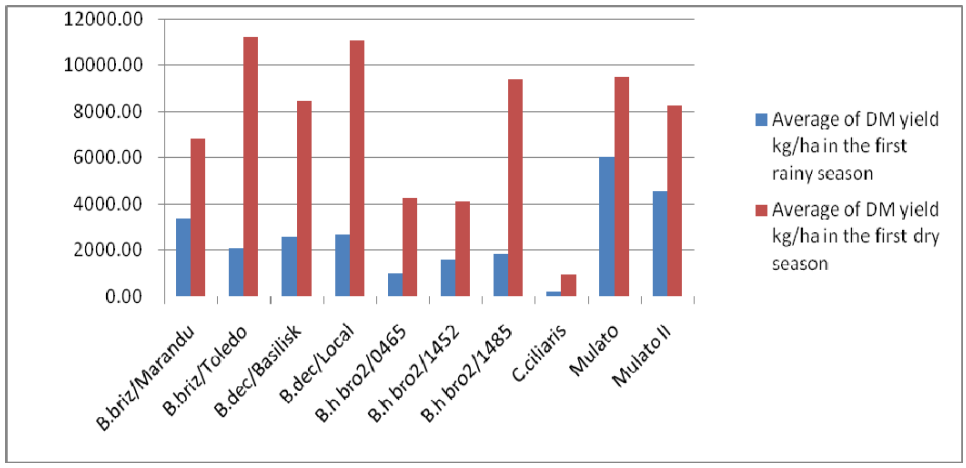

Figure 2: Evaluation of *Brachiaria* cultivars and hybrids for dry matter yield (kg ha^{-1}) during the first rainy and dry seasons in Bugesera (drought) district, Rwanda

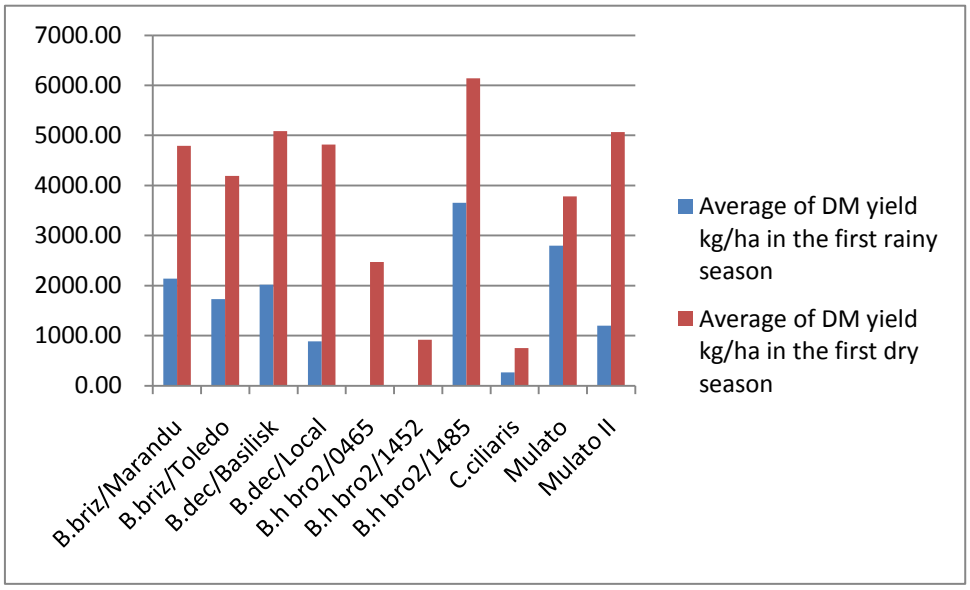

Figure 3. Evaluation of *Brachiaria* cultivars and hybrids for dry matter yield (kg ha^{-1}) for the first rainy and dry seasons in the Nyamagabe (acid soil) district, Rwanda

3.1.3.2 Nutritive value of tested grasses

In the drought-prone environment (Bugesera district), the highest quality grass in terms of crude protein (CP) during the wet season was *Brachiaria* hybrid BR02/1485 (15.69%). There was, however, no significant difference ($p > 0.05$) in the wet season between cv. Marandu, *Brachiaria* hybrid BR02/1452, cv. Mulato and cv. Mulato II (9.83–12.29%) (Table 1), while the control grasses had lower CP content (5.07–6.68%). During the dry season, the CP content decreased for all tested grasses except for *C. ciliaris*, which had slightly higher CP in the dry season than in the wet season in the low rainfall area (5.82% versus 5.07%). In this area during the dry season, cv. Mulato II had the highest CP content (9.85%). It was followed by cv. Marandu and hybrid BR02/1485 which had 8.29 and 8.62% of CP, respectively. The lowest CP content during the dry period in the low rainfall area was found in the two local control grasses *B. decumbens* (indigenous) and *C. ciliaris*, which obtained 6.43 and 5.82%, respectively.

Table 1. Mean crude protein content of tested grasses during the wet and dry seasons in Bugesera (drought) and Nyamagabe (acidic soil) districts, Rwanda.

	Bugesera district		**Nyamagabe district**	
	Crude protein (%)		**Crude protein (%)**	
Treatments	**Wet**	**Dry**	**Wet**	**Dry**
B. brizantha cv. Marandu	9.83^{abc}	8.29^{bcd}	10.91^{abc}	6.69^{cd}
B. brizantha cv. Toledo	7.58^{ab}	7.32^{abc}	12.38^{bc}	7.74^{cde}
B. decumbens cv. Basilisk	6.71^{ab}	7.49^{cbc}	9.43^{ab}	6.41^{bcd}
B. decumbens cv. Local	6.68^{ab}	6.43^{ab}	9.48^{ab}	4.92^{ab}
Brachiaria hybrid BR02/0465	8.20^{ab}	6.73^{abc}	9.21^{ab}	5.77^{abc}
Brachiaria hybrid BR02/1452	10.91^{abc}	6.95^{abc}	8.90^{ab}	4.34^{a}
Brachiaria hybrid BR02/1485	15.69^{c}	8.62^{cd}	12.69^{bc}	7.25^{cde}
Cenchrus ciliaris	5.07^{a}	5.82^{a}	7.88^{a}	5.55^{abc}
Brachiaria hybrid cv. Mulato	11.94^{bc}	7.81^{bc}	11.56^{abc}	7.07^{cd}
Brachiaria hybrid cv. Mulato II	12.29^{bc}	9.85^{d}	14.29^{c}	8.91^{e}
$LSD_{0.05}$	**6.67**	**1.91**	**4.40**	**1.70**

Means in the column followed by the same superscript letter are not significantly different; $LSD_{0.05}$: Least Significant Difference at level of 5%.

During the wet season in the acidic soil area (Nyamagabe district), the highest quality grasses in terms of CP were cv. Marandu, cv. Toledo, Local *Brachiaria*, hybrid BR02/1485, cv. Mulato and cv. Mulato II (10.91–14.29%), while the lowest CP (7.88%) was recorded for *C. ciliaris* (control) (Table 1). During the dry season, the differences in CP were more marked, and. the CP content in all tested grasses declined significantly (Table 1). The lowest CP content was found in *Brachiaria decumbens* (local), *Brachiaria* hybrid BR02/0465, *Brachiaria* hybrid BR02/1452 and *Cenchrus ciliaris*, which obtained 4.92, 5.77, 4.34 and 5.55%, respectively (Table 1). The most marked decrease in quality was recorded for local *B. decumbens*, which decreased from 9.48% CP in the wet season to 4.92% in the dry season.

During the wet season in the low rainfall area (Bugesera district), the calcium (Ca) content was not significantly different ($p>0.05$) for the tested grasses, ranging from 0.89% for *C. ciliaris* to 2.16% for *Brachiaria* hybrid BR02/0465 (Table 2). However, during the dry season, the concentration of Ca differed between treatments. It was found that mean values of Ca in cv. Toledo, cv. Basilisk, *Brachiaria decumbens* (local), *Brachiaria* hybrid BR02/0465, *Brachiaria* hybrid BR02/1452, *Brachiaria* hybrid BR02/1485, cv. Mulato and cv. Mulato II were not significantly different (1.56–2.17%). The lowest Ca content was recorded for *C. ciliaris* (1.10%).

In contrast to the low rainfall area, the Ca content in treatments from the acidic soil area during the wet season showed that cv. Marandu had the highest CA concentration (2.47%), which was significantly different ($p<0.05$) from cv. Basilisk, *Brachiaria* hybrid BR02/0465 and *C*. *ciliaris* (1.3–1.82%) (Table 2). Similarly, during the dry season in the acidic soil area, the mean values of Ca content in cv. Marandu were significantly higher (2.35%) than those found in cv. Basilisk, *Brachiaria* hybrid BR02/0465 and *C*. *ciliaris* (1.51–1.76%). The remaining treatments were not different from cv. Marandu in Ca content (1.89–2.14%). When comparing the two sites, there was no significant difference in Ca content between the wet and dry season (Table 2).

Table 2. Mean values of calcium of tested grasses during the wet and dry seasons in Bugesera (drought) and Nyamagabe (acidic soil) districts, Rwanda.

	Bugesera district		Nyamagabe district	
	Calcium (%)		Calcium (%)	
Treatments	**Wet**	**Dry**	**Wet**	**Dry**
B. brizantha cv. Marandu	1.54^{ab}	1.43^{ab}	2.47^{d}	2.35^{d}
B. brizantha cv. Toledo	1.91^{b}	1.96^{bc}	1.89^{abcd}	1.89^{abcd}
B. decumbens cv. Basilisk	1.73^{b}	1.56^{abc}	1.51^{ab}	1.51^{a}
B. decumbens cv. Local	1.98^{b}	1.97^{bc}	2.20^{cd}	2.20^{cd}
Brachiaria hybrid BR02/0465	2.16^{b}	2.04^{bc}	1.82^{abc}	1.76^{abc}
Brachiaria hybrid BR02/1452	1.72^{b}	1.97^{bc}	2.03^{bcd}	2.11^{bcd}
Brachiaria hybrid BR02/1485	1.81^{b}	1.87^{bc}	2.05^{bcd}	1.99^{abcd}
Cenchrus ciliaris	0.89^{ab}	1.10^{a}	1.30^{a}	1.57^{ab}
Brachiaria hybrid cv. Mulato	2.13^{b}	2.17^{c}	2.31^{cd}	2.13^{bcd}
Brachiaria hybrid cv. Mulato II	1.87^{b}	1.73^{abc}	2.36^{cd}	2.14^{bcd}
$LSD_{0.05}$	**0.74**	**0.69**	**0.60**	**0.58**

Means in the column followed by the same superscript letter are not significantly different; $LSD_{0.05}$: Least Significant Different at level of 5%.

The phosphorus (P) content during the wet season in the low rainfall area was significantly higher for cv. Toledo (0.28%) than for Brachiaria hybrid BR02/1452 (0.11%) and C. ciliaris (0.05%). The mean P content of the remaining treatments were not significantly different from the P content in cv. Toledo (Table 3). In the low rainfall area during the dry season, the only difference in P content was observed between cv. Toledo and the other treatments.

Table 3. Mean values of phosphorus in the treatments during the wet and dry seasons in Bugesera (drought) and Nyamagabe (acidic soil) districts, Rwanda.

	Bugesera district		Nyamagabe district	
	Phosphorus (%)		Phosphorus (%)	
Treatments	**Wet**	**Dry**	**Wet**	**Dry**
B. brizantha cv. Marandu	0.16^{abc}	0.17^{ab}	0.41^{abc}	0.42^{b}
B. brizantha cv. Toledo	0.28^{c}	0.29^{b}	0.29^{ab}	0.29^{ab}
B. decumbens cv. Basilisk	0.18^{abc}	0.20^{ab}	0.39^{abc}	0.39^{b}
B. decumbens cv. Local	0.25^{bc}	0.25^{ab}	0.47^{bc}	0.47^{b}
Brachiaria hybrid BR02/0465	0.21^{abc}	0.21^{ab}	0.41^{abc}	0.35^{ab}
Brachiaria hybrid BR02/1452	0.11^{ab}	0.12^{a}	0.40^{abc}	0.40^{b}
Brachiaria hybrid BR02/1485	0.23^{bc}	0.23^{ab}	0.54^{c}	0.53^{b}
Cenchrus ciliaris	0.05^{a}	0.10^{a}	0.21^{a}	0.11^{a}
Brachiaria hybrid cv. Mulato	0.17^{abc}	0.26^{ab}	0.39^{abc}	0.39^{b}
Brachiaria hybrid cv. Mulato II	0.23^{bc}	0.23^{ab}	0.43^{abc}	0.43^{b}
$LSD_{0.05}$	**0.17**	**0.18**	**0.24**	**0.24**

Means in the column followed by the same superscript letter are not significantly different; $LSD_{0.05}$: Least Significant Difference at level of 5%.

In the acidic soil area, similar values of P content were measured. It was observed that during the wet season, P in grasses was significantly higher in *Brachiaria* hybrid BR02/1485 (0.54%) than in cv. Toledo (0.29%) and *C. ciliaris* (0.21%). The mean values of P content in the rest of the treatments were not significantly different. However, during the dry season in the acidic soil area, the difference of mean values of P content in treatments was only observed between *C. ciliaris* and the rest of treatments. *Cenchrus ciliaris* (control) obtained 0.11% of P, which was the lowest mean value of P recorded in all treatments.

3.1.3.3 Participatory variety selection

In workshops held in both districts, the Bugesera and the Nyamagabe (Plates 5 and 6) farmers ranked and selected accessions and hybrids of *Brachiaria* grass options most suitable to their production system. Among the criteria, drought tolerance was the major criterion mentioned by farmers in Bugesera because of feed scarcity during the dry season. *Brachiaria* hybrid cv. Mulato II was preferred as a forage resisting to drought, followed by *Brachiaria brizantha* cv. Marandu, *B*. hybrid cv. Mulato, and *B. decumbens* cv. Basilisk. Mulato II was appreciated by farmers mainly because of its dry season tolerance since it remained green up to the end of the dry season.

In Nyamagabe district, criteria for farmer's selection were similar to Bugesera. However, farmers in the latter stressed the criterion of persistence, whereas in Nyamagabe they stressed disease resistance. While in Bugesera, drought resistance was the main selection factor for Brachiaria, in Nyamagabe it was acid soil tolerance. Contrarily to Bugesera district, in Nyamagabe district, farmers ranked B. hybrid BR02/1485, cv. Mulato II, and B. decumbens local as superior performers, followed by cv. Basilisk and cv. Toledo.

Plate 5. Farmers in Bugesera participating in ranking of the performance of *Brachiaria* accessions and hybrids compared to the checks

Plate 6. Farmers in Nyamagabe participating in ranking of the performance of *Brachiaria* accessions and hybrids compared to the checks

Across the two districts *Brachiaria* hybrid cv. Mulato II was the preferred option. It has been selected by farmers under the two stress conditions because of its adaptability to these contrasting environments of drought and acid soil stress, producing green forage year round, without any input of fertilizer.

Assessment of the acceptability of new forage options through gender analysis: Preliminary observations indicate that women-headed households put more efforts into innovative technologies than men. This is related to the spread of the zero-grazing system across Rwanda (Plate 7).

Plate 7. A child of the farmer Karimbanya L. in Bugesera district carrying *Brachiaria* grass to feed his dairy cow

4. Monitoring and evaluation of new adopters of *Brachiaria* grass

4.1 Materials and methods

To continue the work with already established farmer groups that were exposed to *Brachiaria* grass options, a survey on the early adoption by farmers was carried out. This survey started by asking farmers who have established *Brachiaria* grass the area that he/she has extended with this new forage option. From there, farmers indicated to new farmers that acquired planting materials of *Brachiaria* grass and to their neighbours.

4.2 Results

In the drought (Bugesera) and acidic soils (Nyamagabe) conditions, monitoring and evaluation of early adoption of the new *Brachiaria* grass options showed the following: among farmers who established *Brachiaria* grass in the acidic soil area, 41.6% of them increased the size of plot to 0.04 ha per farm (Plate 1). In the drought area, 66.6% of farmers who had *Brachiaria* grass increased their plot up to 0.1 ha per farm (Plate2).

Plate 1. Selected *Brachiaria* grass cultivars (cv. Mulato II, cv. Basilisk) were multiplied on a large plot by the farmer Tumushime Monique in Bugesera district (drought prone).

Plate 2. Selected *Brachiaria* grass cultivars (cv. Marandu, cv. Mulato II) were multiplied on large plot by the farmer Halerimana Theoneste in Nyamagabe district (acid soils).

During the assessment of farmers who adopted *Brachiaria* grass, it was observed that in the drought area, some farmers came and took planting materials from their neighbours without prior consultation. This has been a constraint to define exactly the number of the new *Brachiaria* adopters. This situation was similar to the acidic soil area where new farmers could acquire *Brachiaria* grass without asking to

the owners. However, five farmers who got *Brachiaria* without asking the owner in the acidic soil area, have been identified and visited; all of these planted the new forage options on erosion control bounds (Plate 3).

Plate 3. Large plot of *Brachiaria* grass of the cooperative KOABRANYA.

Employing the knowledge obtained with the evaluation of *Brachiaria* grasses, a number of farmers in both contrasting districts, formed cooperatives with the objective of multiplying and selling planting materials of *Brachiaria*. In Bugesera district (drought area), the established cooperative has 15 members. It is called "Cooperative of crop-livestock owners aiming at multiplying *Brachiaria* grass in Bugesera (KOABBU: acronym in local language)". This cooperative has multiplied *Brachiaria* hybrid Mulato II, cv. Toledo, hybrid BRO2/1485 and cv. Marandu on 0.35 ha. In the acidic soil area (Nyamagabe district), the cooperative formed has 12 members. It is named "Cooperative of growers of *Brachiaria* of Nyamagabe (KOABRANYA: acronym in local language)". This cooperative has started to multiply *Brachiaria* hybrid cv. Mulato II, cv. Basilisk, cv. Toledo and cv. Marandu on a plot of 0.4 ha (Plate 4) with the aim to increase it by the next cropping season (March-April 2010).

Plate 4. *B. brizantha* cv. Toledo planted on contour ridges between crops in acidic soil area.

5. Training course on improved forage production and utilisation in Rwanda

A training workshop of technicians from ISAR and extension workers from NGOs on "Improved forage production and utilisation in Rwanda" was held at ISAR Rubona Station, 22-26 February, 2010. The

main trainers were from CIAT and ISAR. The objective of this training was to empower technicians and extension workers in the domain of forage and animal feeding in Rwanda. The course was well attended by 22 participants, all men, being technicians from the government institutions ISAR, RARDA/MinAgri and RADA/MinAgri, as well as NGOs in Rwanda (Heifer International, Send-a-Cow). The decision who to send was by the sending institution. Participants came from all regions of Rwanda, which will improve knowledge transfer within the country (Plate 5).
At the beginning of the course, participants' expectations were gathered, which helped the trainers to achieve the objectives of the training. The course covered the following themes:

- General introduction to forages, terms used and forage development (Maass)
- Feed resources available in Rwanda (Mutimura)
- Feed requirements of livestock (Myambi)
- Crop residues as feed for livestock in Rwanda (Ebong)
- An introduction to participatory forage research with farmers (Maass and Mutimura)
- An introduction to the SoFT database (Maass)
- Adaptation of forages to production systems and agro-ecology (Maass)
- Forage seed production (Maass and Mutimura)
- Identifying niches for improved forages in Rwanda (Maass et al.)

Plate 5. Group photo in a field during training course at ISAR Rubona forage plots.

Some of the contents was provided by lectures, other by group work or interactive discussions with the participants. Theory and practical course were designed to meet the needs of the participants who daily work with smallholder farmers. The training will help them to achieve some issues related to animal feeds and feeding at smallholder farmer level, especially in drought and aluminium toxicity areas of Rwanda. Certificates of attendance and different training manuals like booklets, and all teaching materials used were provided on a CD to every participant.
Participants were very interested and open to the course contents, but already from the initial collection of their expectations, it was clear that some desired contents would not be covered, e.g., forage conservation. There were several intensive debates and, generally, very good participation. Most participants had a veterinary technician background and, thus, did not know too much about plants. For most of them, it was new to see how variable forage plants can be; and probably, it was the first time ever that they consciously observed forage grasses and legumes. For most participants, it was also eye-opening where (spatial niche) and when (temporal niche) in a production system forages can be included. Visits to a nearby farm, where the farmer and his wife kindly answered many questions, and to the small plots used for demonstration at ISAR-Rubona where useful for interaction with the real world. However, truly practical exercises how to manage forages would be even more useful for persons that have not had much experience with plants.

Most of the course was either held in French or in Kinyarwanda (local language), which, language-wise, was a challenge because not all participants/instructors would be able to follow in both languages. So occasionally, English was also used in order to translate some of the findings. Nevertheless, for some lively debate, it was indispensable to let people express themselves in their best ways. Various participants appeared not to have much practice with computers so that the provision of electronic materials may not be too useful for them.
In a final discussion among all participants, the following themes were identified that needed more training both in the class room and in practice:

- How to follow a protocol of evaluating forages with a farmer, with practical application;
- Forage conservation (hay and silage), especially in practice with farmers;
- More details about rations and how they can be improved when critically analyzing a farmer's practices.

This training course was useful and all participants showed their gratitude due to the knowledge and skills obtained and agreed to share them with smallholder farmers. Since the course was held, one participant reported back two months later that he had established trials with new forages.

Training course instructors

- Brigitte Maass, Dr. sc. agr., Forage Agronomist at CIAT, Nairobi, Kenya
- Mupenzi Mutimura, MSc, Forage Agronomist at ISAR Karama Research Station
- Celestin Myambi, MSc, Animal Nutritionist at ISAR Rubona Research Station
- Cyprian Ebong, PhD, Animal Nutritionist at ISAR Rubona Research Station (originally from NARO in Uganda, recently stationed in Rwanda)

Abbreviations and acronyms

Abbreviation/ acronym	Details
ISAR	National Agricultural Research Institute (Institut des Sciences Agronomiques du Rwanda), Rwanda
MinAgri	Ministry of Agriculture and Animal Resources
NARO	National Agricultural Research Organization, Uganda
RADA	Rwanda Agriculture Development Authority
RARDA	Rwanda Animal Resources Development Authority
SoFT	A tool for Selection of Tropical Forages (http://www.tropicalforages.info/)

6. Conclusions

Gender analysis carried out in the two contrasting districts of Rwanda showed the common and diverse activities related to livestock rearing between men and women. Because of zero grazing found in the two districts, both genders shared livestock activities. However, due to the Rwandan culture, activities like milking cows, construction of cattle sheds, and treatment of animal diseases are confined to men and boys. This could potentially hinder the development of livestock production in confinement because a household that does not have a boy or husband may not raise cows for milk production.
Wealth ranking was shown to have an impact on livestock management. For example, of the five wealth categories the top two ('very rich' and the 'rich') were characterized by the possession of cows and land. These two characteristics are important in the areas where there are dense populations like in the Bugesera and Nyamagabe districts. This is because where small plots are over-exploited, agricultural production can only be increased if there is addition of manure. Thus, 'very poor' to 'moderately poor' households will have plots prone to low production.
Animal feed resources identified in the Bugesera and Nyamagabe districts showed scarcity during the year. Although farmers identified thirteen feed resources in the Bugesera district and twenty-one in the Nyamagabe district, their availability during the year was limited. For example, in the Nyamagabe district, low quality feed such as *Commelina benghalensis* and banana stems were fed to animals by up to 50% of the farmers during the rainy and dry seasons. In the Bugesera district, low quality banana stems were used by 80% of farmers to feed their cows during the dry season. The grasses like Napier grass that should constitute most of the ruminant diet were used at 25% during the dry season in the Nyamagabe district. In addition, the use of low nutritive value feeds (e.g. banana stems, leaves of

trees like *Albizia* spp. or *Ficus* spp.) confirmed the need for intervention in forage options of the study areas. Forage crops that are of good quality and can adapt to the particular environmental constraints found in each district will be important. For example, in Bugesera district, the forage crops should be tolerant to the long dry period, whereas in Nyamagabe district, they ought to be tolerant to the combination of soil acidity and aluminium toxicity.

On-farm trials were established to enable farmers to evaluate different cultivars and hybrids of improved *Brachiaria* to increase forage production under these conditions. The most preferred variety of *Brachiaria*, which was selected by farmers across the two sites, was cv. Mulato II. It was selected by the farmers because of its adaptation to the two contrasting sites and its production of green forage (leaves and stems) during every season of the year.

The chemical analyses of each grass used in this study, indicated that the hybrid BR02/1485 and cv. Mulato II had the highest nutrient content (CP >11 %) in both sites. The control entries had the lowest CP contents, which were 5.44 and 6.56% for *C. ciliaris* and local *Brachiaria* grass, respectively. The mineral content varied according to species and sites, however, they were rarely significantly different among the different cultivars and hybrids. The control grass (*C. ciliaris*) had the lowest minerals (Ca <1.4% and P<0.16%) compared to the rest of treatments in both sites. Since the minimum nutrient requirements for a late stage pregnant cow are 11% CP, 0.37% Ca and 0.26% P, this study indicates that neither the control grass *C. ciliaris* nor the control grass *B. decumbens* (local) can meet these requirements of CP, which is a crucial nutrient in animal nutrition. However, cv. Mulato II, selected by the farmers can provide adequate nutrients.

The most productive grass in terms of DM in the low rainfall area was cv. Toledo (5.71 t ha^{-1}) and in the acidic site, the hybrid BR02/1485 (5.95 t ha^{-1}). However, the highest DM producer in the low rainfall was not significantly different ($p>0.05$) from the local *Brachiaria* (5.6 t ha^{-1}). In the acidic soil area, the two control grasses (local *Brachiaria* and *C. ciliaris*) had lower DM yields (3.72 and 1 t ha^{-1}, respectively) compared to the top producer in this site. The high nutrient content of the improved *Brachiaria* grasses is likely to increase milk yield, which will encourage farmers to multiply these grasses to a large scale.

Monitoring and evaluation of *Brachiaria* grass options in the contrasting environments of Rwanda has enhanced the awareness of smallholder farmers about new forage alternatives in both drought prone and acidic soil environments. The interest of farmers for *Brachiaria* is increasing as reflected by replacement of the commonly used forage grass (Napier) by *Brachiaria* on erosion control ridges. At the same time, farmer cooperatives are set up based on multiplying planting materials of *Brachiaria* for feeding their animals and selling forage to other dairy farmers to generate income.

7. Publications

Mutimura, M. 2009. Evaluation of improved *Brachiaria* grasses in low rainfall and aluminium toxicity prone areas of Rwanda. MSc thesis. University of Kwazulu-Natal, Pietermaritzburg, South Africa, 179 pp.

Mutimura, M. and Everson, T.M. 2009. Assessment of livestock feed resource-use patterns in low rainfall and aluminium toxicity prone area of Rwanda. In: Grassland Society of Southern Africa (GSSA), 44th Annual Congress: Meeting Rangeland, Pasture and Wildlife Challenges in a Changing Landscape. 20-25th July 2009. University of South Africa, Roodepoort, South Africa. Book of Abstracts, pp 82–83.

INTA-NICARAGUA

Agronomic and participatory evaluation of *Brachiaria* grasses tolerant to drought and aluminum toxicity in Nicaragua

Contributors: M. Mena, R. van der Hoek, L. Urbina, C. Gutiérrez, G. Arguello and A. Schmidt

Rationale

Livestock represents one of the most important activities for Nicaraguan economy by means of its contribution to GDP, export, income generation and food production. But productivity of the large majority of livestock farmers is still low. At least 90% of livestock producers are small and medium scale farmers, and they own 65% of national herd. One of the most important limiting factors in livestock production is animal feed, both in quantity and quality, especially during the dry season which in turn represents a major challenge due to climate change. Although many efforts have been made to develop new forage options, results and impact on livestock production and well-being of rural families have not been satisfactory. Especially to contribute to the well-being of farmers in less

privileged areas, there is a need for research with other species and accessions that are better adapted to low rainfall and irregular rainfall patterns.

In order to contribute to develop options that help to overcome the lack of animal feed, the Nicaraguan national agricultural research institute (INTA) carried out together with CIAT activities within the framework of the project "Fighting drought and aluminium toxicity: Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and *Brachiaria* for the tropics", financed by BMZ. The objective was to evaluate the adaptation of new forage grass materials (*Brachiaria*) in the tropical dry zone in Nicaragua and to develop together with the farmers new options that contribute to improving feed availability as well as quality.

INTA's activities were addressed to contribute to the Output 1 in this project: Rural benefits enhanced in the target areas of tropical Africa and Central America by involving women and small-scale farmers as decision makers and co-researchers in the common bean and *Brachiaria* product development process. So a field trial with farmers was carried out to do agronomic and participatory evaluation of newly introduced germplasm of *Brachiaria* in a site with drought stress and low rainfall located in the municipality of Diriamba, next to the Pacific Ocean in Nicaragua. Also, at the end of the project a workshop with technicians, farmers and representative of governmental and non-governmental organizatios was held.

1. Agronomic evaluation and PVS on forages

Materials and Methods

A farmer was selected in the municipality of Diriamba, Carazo department. An area of approx. 625 m^2 was planted with five hybrids (three new and two recently introduced commercial) and three cultivars of *Brachiaria spp* using botanical seed supplied by CIAT in furrows at distances of 0.5 m and 0.2 m between plant holes. Initial weeding took place with a *machete* followed by one round of ploughing. Due to heavy rains during the first week after planting, that dragged seeds and covered them with soil. Because of Spittlebug and Rhizoctonia infestations, at the onset of the rains in 2007 most of the plots were replanted with vegetative material. Germination rates and plant vigor were evaluated visually using a scale from 1 to 5. A germination rate of 1 meant that there were only plants in less than 20% of the plant holes showing a disperse distribution, 5 meaning that in more that 90% of the plant holes germination took place resulting in a good distribution in the whole plot. In plant vigor, 5 corresponded to plots with the best plants, with good physical characteristics and a high number of leaves or shoots. Additionally, forage dry matter production was measured cutting herbage in a 2 square meter frame placed randomly within each plot at five weeks intervals during the rainy season and at six weeks in dry season. After collecting samples, the whole plot was cut to allow homogenous regrowth for the next cut. Samples were taken in order to determine dry matter content for each grass (Photo 1).

Photo 1. Taking samples at the trial site in Carazo

Results and Discussion

Establishment: The results of the measurements between 2 and 7 weeks after planting showed generally good germination rates (Figure 1) resulting in high plant densities in most plots at 7 weeks after planting, except for some plots with the *Brachiaria* hybrids 36087, BR02/1452 and CIAT 36061 that counted only 25, 27 y 13 plants per plot, respectively. This was due to heavy rains during the first week after planting that dragged seeds and covered them with soil.

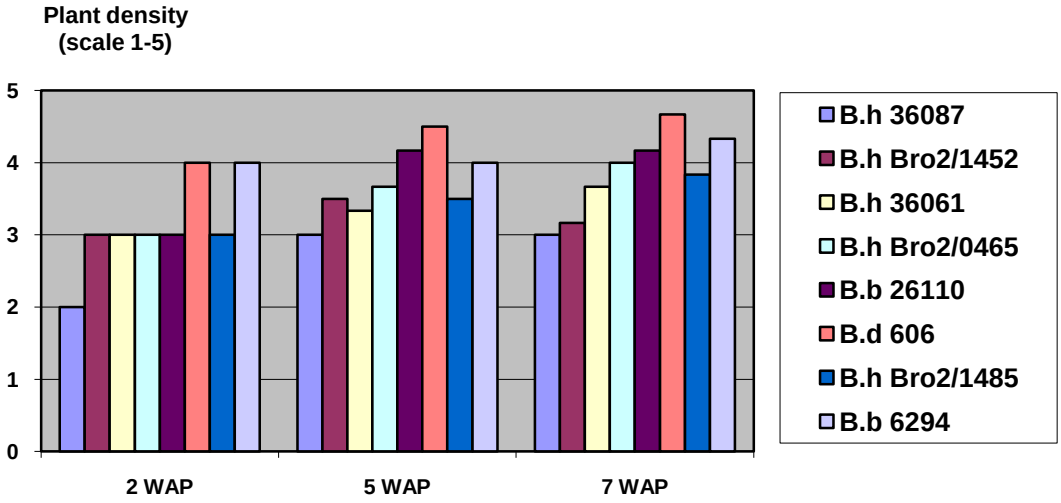

Figure 1. Germination/plant density of 8 *Brachiaria* accessions during the establishing phase between 2 and 7 weeks after planting (WAP) at a site in Carazo, Nicaragua, 2006.

The accession *B. decumbens* CIAT 606 showed a plant vigour of 5 at 5 weeks after planting, together with the accession *B. brizantha* CIAT 26110; however, at 7 weeks after planting the first showed a better development and higher vigor than the other accessions. *B*. hybrids 36087 and BR02/1452 performed less during the establishing phase (Figure 2).

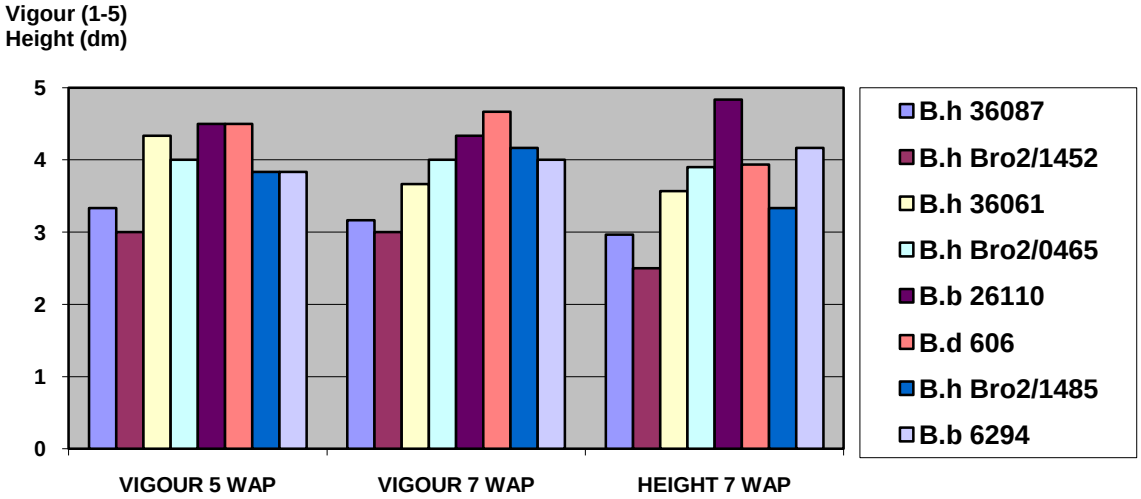

Figure 2. Plant vigor and height of 8 *Brachiaria* grasses during the establishing phase between 2 and 7 weeks after planting (WAP) at a site in Carazo, Nicaragua, 2006.

Soil cover and biomass production: Soil cover and biomass production of most accessions increased in the course of the first year (2007). Exceptions was the *Brachiaria* hybrid BR02/1452, which also showed low vigor. Especially, cv. Mulato II (CIAT 36087) showed a remarkable increase in soil cover from 60% to almost 90% (Figure 3) and this was also reflected in an increase in biomass production from 3,3 to 7,0 t DM/ ha, the latter being superior to all accessions at one year after planting. In 2008,

general performance was less than in 2007 (Figure 4). This was mainly due to a long dry spell and probably due to the infestation of Spittlebug and *Rhizoctonia*. *Brachiaria* hybrids cv. Mulato (Photo 2) and BR02/0465 (Photo 3) were performing relatively better than in the previous year, also in comparison with most other grasses (except for cv. Toledo).

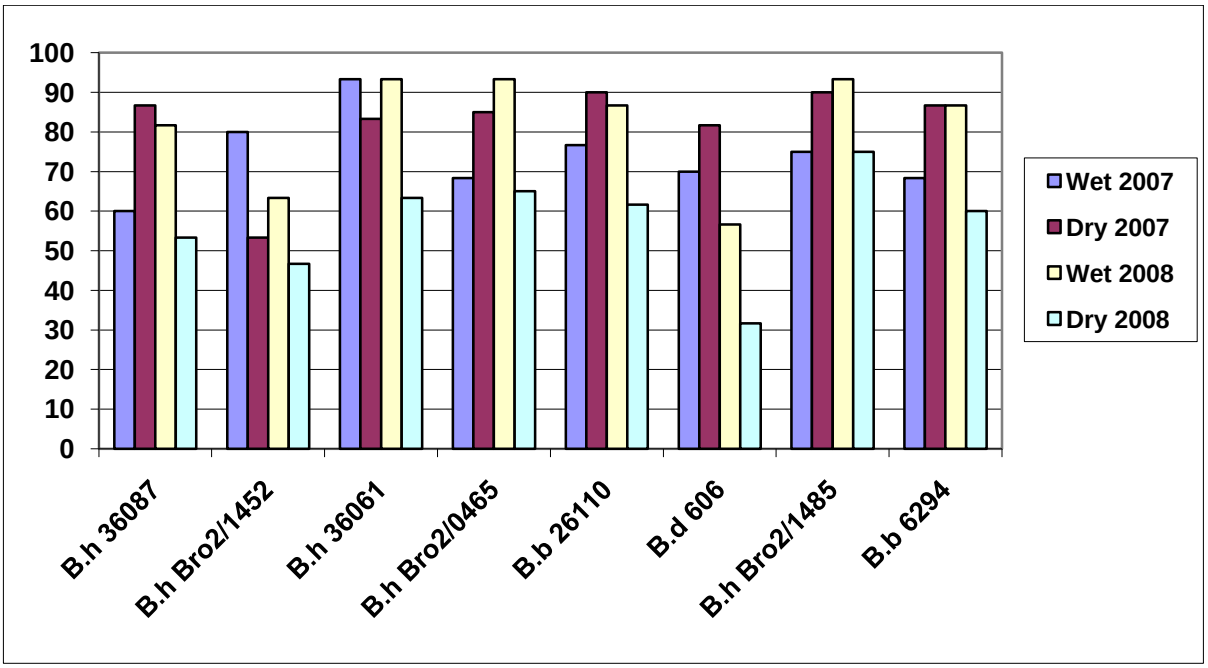

Figure 3. Average soil cover (%) of 8 *Brachiaria* grasses in wet and dry season at a site in Carazo, Nicaragua, 2007-2008.

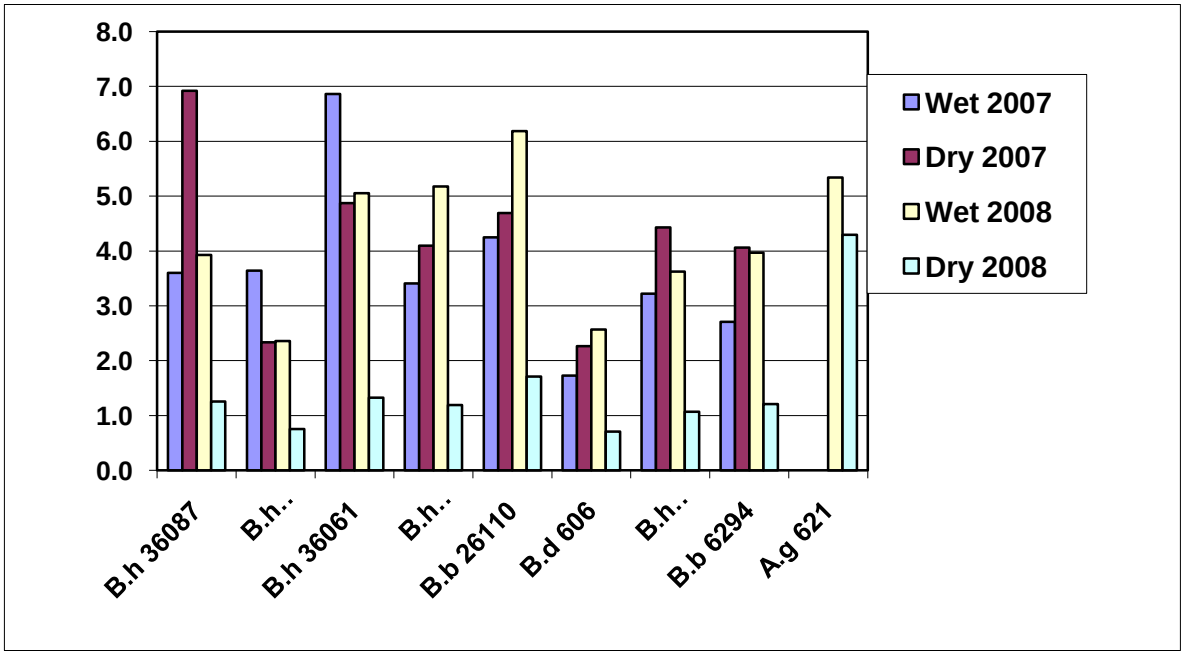

Figure 4. Average forage biomass production of 8 *Brachiaria* grasses in wet and dry season at a site in Carazo, Nicaragua, 2007-2008.

Despite that all plots of the *Brachiaria* hybrid BR02/1452 were replanted during the rainy season in 2007, this hybrid showed a decrease in soil cover to the end of the year leading to the lowest value of all ($p<0.05$). This was due to plant mortality related to excessive soil moisture after heavy rains.
Most findings were confirmed by the participatory evaluation with farmers with highest ranks of preference for cv. Mulato II, *A. gayanus* and the *Brachiaria* hybrid BR02/0465. Most important criteria used by farmers were forage biomass production, drought adaptation and texture of leaves and stems (palatability).
Conclusions: In general, all accessions established well, especially *B. decumbens* CIAT 606 for its highest germination and plant vigor scores. Differences between grasses in soil cover and forage biomass production were significant and generally more pronounced in the dry season. Among the 8 *Brachiaria* genotypes evaluated on-farm for their adaptation to combined stress of drought and aluminum toxicity, *Brachiaria* hybrid cv. Mulato II (CIAT 36087) and *B. brizantha* cv. Toledo (CIAT 26110, Photo 4) performed well in both dry and wet season based on soil cover and forage biomass production. Within the second year, a notable recovery was observed with the hybrid BR02/0465 (Photo 3), which has good soil cover and biomass production in both wet and dry season, and because of this the field trial needs to be continued for monitoring the performance of different grasses.

Photo 2. *Brachiaria* hybrid (Mulato) showing best overall performance

Photo 3. *Brachiaria* hybrid (B02/0465): most promising new material

Photo 4. *Brachiaria brizantha cv.* Toledo : good overall performance

2. PVS on forages
Most findings were confirmed by the participatory evaluation with farmers with highest ranks of preference for cv. Mulato II and the Brachiaria hybrid BR02/0465. Most important criteria used by farmers were forage biomass production, drought adaptation, texture of leaves and stems (which was related with palatability and forge intake by animals) and tolerance to pest and diseases (mainly spittlebug and *Rhizoctonia*)

Workshop: "Dry season feeding – problem at present and challenge for the future"
At the end of the project a workshop was organized at national level with some 60 farmers, technicians, researchers (INTA, CIAT, UNA-National Agricultural University, and others) and representatives of farmer organizations. The objectives of this workshop were: (i) presentation of project results; (ii) presentation of experiences in dry season feeding in Nicaragua (last 5 year); and (iii) discussion of results, formulation of a strategy for the coming years (Photo 5). The themes discussed included: (i) a general description of the situation constraints and opportunities); (ii) the (potential) contribution of drought adapted forages: both grasses (with a focus on *Brachiaria*) and legumes (use of legumes in combination with crop residues, leguminous trees/shrubs in agroforestry systems) to maintain/increase animal production in the dry season and/or increase soil fertility; (iii) conservation of forages (silage, hay); and (iv) different climate change scenarios and its effect on forage production.

Photo 5. Presentation and discussion of working group results during the final workshop

ANNEX 2

Project Title

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

Project Supported by
Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)

Executed by
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in collaboration with
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Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics

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Physiological and molecular mechanisms

Output 2: Physiological mechanisms characterized in contrasting parents, interactions defined, and screening methods developed, for: root elongation, water and nutrient uptake and transport in roots and effects on shoot growth under individual or combined stress factors of drought stress and Al toxicity

Common bean

Brachiaria

ANNEX-2

Physiological and molecular mechanisms

Output 2: Physiological mechanisms characterized in contrasting parents, interactions defined, and screening methods developed, for: root elongation, water and nutrient uptake and transport in roots and effects on shoot growth under individual or combined stress factors of drought stress and Al toxicity

Common bean

1. Aluminum toxicity and resistance in *Phaseolus vulgaris* L. - physiology drives molecular biology

Contributors: W. J. Horst, A. F. Rangel, D. Eticha, M. Ishitani and I. M. Rao

In common bean (*Phaseolus vulgaris* L.) aluminium (Al) inhibits root elongation not only when applied to the transition zone but also to the elongation zone. After a very Al-sensitive initial response (0-4 h), acquired genotype-specific Al resistance (8-24 h) is related to the sustained release of organic acid anions, particularly citrate from the root apices. This requires both the expression/activation of a citrate permease and the maintenance of the cytosolic citrate concentration through up-regulation of citrate synthesis and down-regulation of its degradation. Proteomic and transcriptomic studies focussed on the dynamics of the expression of Al resistance in an Al-resistant genotype allowed to pinpoint some of the decisive genes.

Common bean is the most important food legume for more than 300 million people, most of them in the developing world. About 40% of the bean-growing area in Latin America, and in central, eastern, and southern Africa is affected by aluminium (Al) toxicity resulting in yield reduction up to 60%. Correction of acidity-related soil constraints using lime and phosphate fertilizers are beyond the capacity of resource-poor farmers. They need to make use of the genetic variation existing for adaptation to acid soils and Al resistance among common bean genotypes. Knowledge on the physiological and molecular mechanisms of Al toxicity and Al resistance can contribute to the development of rapid and reliable screening procedures needed for accelerated conventional and molecular breeding for Al resistance. This paper focuses initially on the temporal and spatial effects of Al on root growth, Al accumulation and cellular localization, and Al exclusion mediated by the release of organic acid anions.

In agreement with Cumming et al. (1992) our results comparing two genotypes of common bean differing in Al resistance clearly corroborated that Al resistance is an Al-inducible trait. Root elongation of both genotypes was severely inhibited during the first 4 h of Al treatment. The analysis of spatial growth profiles revealed that the initial inhibition of root elongation by Al resulted from a generalized effect along the entire elongation zone (EZ). This was reflected by a reduced maximal rate of relative elongation, without changing the shape or the length of the entire EZ. Localized application of Al to specific zones of the root apex showed that in both genotypes application of Al to the transition zone (TZ) resulted in root-growth inhibition to the same extent as if the whole root tip would have been treated with Al. These results confirmed previous studies with maize, reporting that the TZ is the most Al-sensitive apical root zone. However, in contrast to maize (Kollmeier et al., 2000) application of Al to the EZ also reduced root growth in both common bean genotypes, though to a lesser extent than when applied to the TZ. After the initial growth inhibition, both Al-resistant and Al-sensitive genotypes showed gradual recovery. However, this recovery continued in genotype Quimbaya (Al-resistant) until the root-elongation rate reached the level of the control (without Al), while the genotype VAX-1 (Al-sensitive) was increasingly damaged by Al after 12 h of Al treatment.

The observed Al-induced inhibition of root elongation corresponded with an enhanced Al accumulation in the TZ owing to higher pectin contents of this zone. Additionally, the higher initial Al accumulation of in the root apex of Quimbaya compared to VAX-1 could be related to its higher content of unmethylated pectin and thus higher negativity of the cell wall (CW). Aluminium treatment enhanced the root-tip pectin-content in both genotypes and decreased its degree of methylation thus enhancing the overall negativity of the CW.

Results from studies on the binding state and cellular distribution of Al in the root apices during the initial inhibition of root elongation and subsequent recovery in Quimbaya revealed that the root elongation-rate was significantly negatively correlated with the free apoplastic and the stable-bound, not citrate-

exchangeable cell-wall Al representing the most important Al fraction in the root apex (80%), but not with the symplastic and the labile-bound, citrate-exchangeable cell-wall Al. It is concluded that the induced and sustained Al resistance in the Al-resistant Quimbaya is mediated by reducing the stable-bound Al in the apoplast thus allowing cell elongation and division to resume. Greater accumulation of Al in the symplastic fraction in Quimbaya might by explained by the greater cell volume and thus vacuoles that are typical for large seeded genotypes of Andean origin in comparison to the small seeded genotypes of Mesoamerican origin such as VAX-1.

The initial genotype-independent Al injury was related to the absence of citrate exudation from the root tips in both genotypes in spite of high citrate contents in the root apices particularly in Quimbaya. Thereafter (5-9 h), in both genotypes recovery of root elongation was related to an Al-enhanced exudation of citrate typical for pattern-II type Al-induced release of organic acid anions (Ma et al., 2001). Aluminium-enhanced citrate exudation has been previously implicated in Al resistance of common bean (Miyasaka et al., 1991; Mugai et al., 2000; Shen et al., 2002) and in soybean (Silva et al., 2001; Yang et al., 2001). This citrate release requires the activation or expression of an organic anion permease in the plasma membrane and is initially mainly derived from the internal organic acid pool. In genotype VAX-1 citrate efflux could be sustained during the intitial recovery period by a down-regulation of the activity of NADP-isocitratedehydrogenase (ICDH) thus reducing the cytosolic turnover of citrate and a low but constant citrate synthase (CS) activity. In Quimbaya, the initial citrate efflux was sustained by both a lower NADP-ICDH activity and a greater internal citrate pool in spite of a decreased CS activity. Genotype Quimbaya was able to maintain an enhanced citrate exudation and to restore the internal organic acid pool by a recovery of the CS activity after 24 h Al treatment and a constant high level of phosphoenol pyruvate carboxylase (PEPC) activity thus remaining independent of the supply of assimilates through glycolysis as indicated by a lack of response of ATP-phosphofructokinase (ATP-PFK) a key enzyme channeling assimilates into the tricarboxylic acid (TCA) cycle. This led to decreasing root-tip Al contents, and thus recovery of root growth. This response suggests a similar regulation as described for soybean (Ermolayev et al., 2003). In genotype VAX-1 the temporal recovery from initial Al injury through enhanced release of organic acid anions could not be sustained because of the inability to maintain PEPC activity at a higher level. A greatly stimulated ATP-PFK activity under Al stress in VAX-1 may reflect an enhanced need for C skeletons through glycolysis in order to cope with the release of organic acid anions which, however, could not be met.

Based on this detailed physiological characterization we decided to focus further studies aiming at a better understanding of the molecular basis of Al toxicity and resistance particularly on the initial Al-induced inhibition of root elongation (0-4 h Al treatment) and the acquisition of Al resistance (4-24 h Al treatment) in the Al-resistant genotype Quimbaya. Through a proteomic approach using 2D IEF / SDS PAGE and 1D high resolution SDS PAGE followed by Nano-LC-MS/MS peptide analysis, and a transcriptomic approach through the construction of a differential cDNA library using the “Suppression Subtractive Hybridization” (SSH) system, we were able to identify a range of genes that were up and down regulated during the initial Al-stress perception and the acquisition of Al resistance in an identical genetic background. The expression of genes related to citrate membrane-transport and maintaining cytosolic citrate concentrations were quantified using qRT-PCR that allowed us to pinpointing some of the decisive genes involved in the build up of Al resistance in an Al-resistant common bean genotype. The study demonstrates that particularly in plants species with largely unsequenced genome and inaccessible to reverse genetics, an in-depth physiological understanding is a prerequisite for a molecular understanding of Al toxicity and resistance.

References:

Cumming JR, Buckelew Cumming A, Taylor GJ (1992) Patterns of root respiration associated with the induction of aluminium tolerance in *Phaseolus vulgaris*. Journal of Experimental Botany 43: 1075-1081.

Ermolayev V, Weschke W, Manteufel R (2003) Comparison of Al-induced gene expression in sensitive and tolerant soybean cultivars. Journal of Experimental Botany 54: 2745-2756.

Kollmeier M, Felle HH, Horst WJ (2000) Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? Plant Physiology 122: 945-956.

Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends in Plant Science 6: 273-278.

Miyasaka SC, Buta JG, Howell RK, Foy CD (1991) Mechanism of aluminum tolerance in snapbeans: Root exudation of citric acid. Plant Physiology 96: 737-743.
Mugai EN, Agong SG, Matsumoto H (2000) Aluminium tolerance mechanisms in *Phaseolus vulgaris* L.: Citrate synthase activity and TTC reduction are well correlated with citrate secretion. Soil Science and Plant Nutrition 46: 939-950.
Shen H, Yan X, Wang X, Zheng S (2002) Exudation of citrate in common bean in response to aluminum stress. Journal of Plant Nutrition 25: 1921-1932.
Silva IR, Smyth TJ, Raper CD, Carter Jr. TE, Rufty TW (2001) Differential aluminum tolerance in soybean: An evaluation of the role of organic acids. Physiologia Plantarum 112: 200-210.
Yang ZM, Nian H, Sivaguru M, Tanakamaru S, Matsumoto H (2001) Characterization of aluminium-induced citrate secretion in aluminium-tolerant soybean (*Glycine max*) plants. Physiologia Plantarum 113: 64-71.

2. Short and medium term root-growth responses to aluminium in common bean

Contributors: Andrés F. Rangel, I. M. Rao and Walter J. Horst

Rationale: Toxicity of Al in acid soils in the tropics is a serious problem and amending soils with lime is difficult and prohibitively expensive. Common bean needs significant improvement in Al resistance to reduce farmer dependence on lime and fertilizer. Field screening of 5000 germplasm accessions and breeding lines in Al-toxic soils with and without lime (65% Al saturation) indicated significant genotypic variation in seed yield. Likewise, significant genotypic differences for Al resistance have been found in nutrient-solution based screenings using inhibition of root elongation after 36 h at 20 µM Al supply as parameter for Al injury. Aluminium-induced callose formation is an indicator of Al-sensitivity and a reliable parameter for the classification of maize genotypes for Al resistance. Although, short-term Al supply (4 h) led to maximum accumulation of callose in common bean, no relationship was observed between callose formation and root growth inhibition, indicating the limitation for the use of callose as a screening tool for Al resistance. The resistance to Al in common bean was proposed as an inducible trait. If this is true, a period of stress is required before a resistance mechanism is "switched on". In fact, the exudation of citrate in common bean has been reported as a mechanism of Al resistance. The present work aimed to understand the kinetics of Al-induced inhibition of root elongation and Al-accumulation in relationship with the dynamic of citrate exudation in an effort to identify plant traits related to Al resistance in order to develop efficient screening procedures for genetic enhancement of common bean.

Materials and Methods: Three-days old seedlings of an Al-sensitive genotype (VAX-1) and an Al-resistant genotype (Quimbaya) were grown in nutrient solution containing 5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 under controlled environmental conditions. After pH adjustment to 4.5 ± 0.1, plants were exposed to 0 or 20 µM Al for up to 24 h. Root length was measured through marking of the main root 3 cm behind the root tip at the beginning of the treatment. Root Al content was determined by GFASS (Analytical Technologies Inc., Cambridge, UK) after wet digestion. For the collection of citrate exuded from root apices, 10 intact 5-days-old seedlings were bundled and the tips (1 to 2 cm) were incubated for 2 h (during the time of Al exposure up to 25 h) in 10 ml of treatment solution with or without 40 µM Al. Citrate content in exudates and root tips was analyzed using isocratic HPLC (Kroma System 3000; Kontron Instruments, Munich).

Results: Root growth of both genotypes (Al-resistant and Al-sensitive) was severely inhibited during the first 3 to 4 h of Al treatment (Figure 1). Thereafter, both cultivars gradually recovered. However, this recovery continued in genotype Quimbaya until the root elongation rate reached the level of the control (without Al) while the genotype VAX-1 was increasingly damaged by Al after 12 h of Al treatment. After a short recovery period, root elongation of VAX-1 was increasingly inhibited up to 80% compared to the controls after 24h Al treatment. Enhanced inhibition of root elongation during up to 4 h Al treatment was related to high Al contents in root tips of both genotypes (Figure 2). Al contents in root tips were even higher in Quimbaya than in VAX-1. Recovery from Al stress was reflected by decreasing Al contents. After 24 h Al treatment, the differences between the two genotypes observed in root growth were clearly reflected by much higher Al contents in root tips of VAX-1 compared to Quimbaya.

Al induced citrate exudation from root tips showed a lag phase of 3 h (VAX-1) to 4 h (Quimbaya). Thereafter, citrate exudation increased to a higher rate and remained on that high level in Quimbaya whereas it gradually declined in VAX-1 leading to a large difference between the two genotypes after 24 h Al treatment (Figure 3). Independent of the Al treatment, Quimbaya showed higher citrate content in the root tips compared to VAX-1. Citrate content in root tips of both genotypes was slightly increased during

the first 3 to 5 h of Al treatment (Figure 4). After a marked decrease of citrate content in both genotypes, it gradually recovered in Quimbaya by reaching the level of the control value while VAX-1 showed a gradual decline until it was not practically detectable with the HPLC.

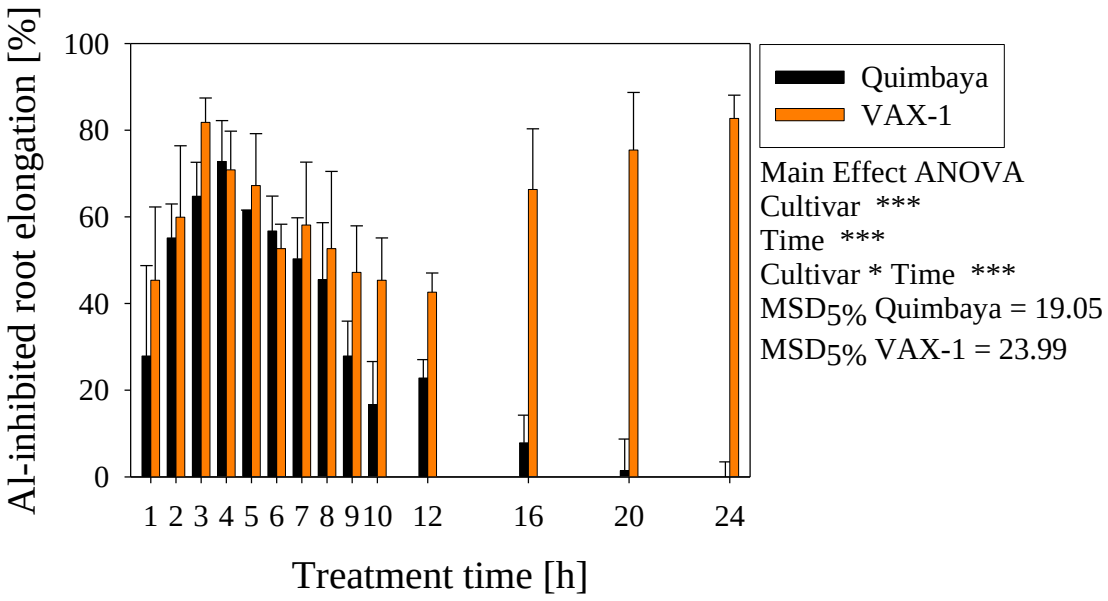


Figure 1. Inhibition of root elongation of two common bean genotypes (Al-resistant Quimbaya and Al-sensitive VAX-1) grown in a solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 with or without 20 µM Al for up to 24 h, pH 4.5. Bars are means ± SD of eight replicates. *** Significant at $P < 0.0001$.

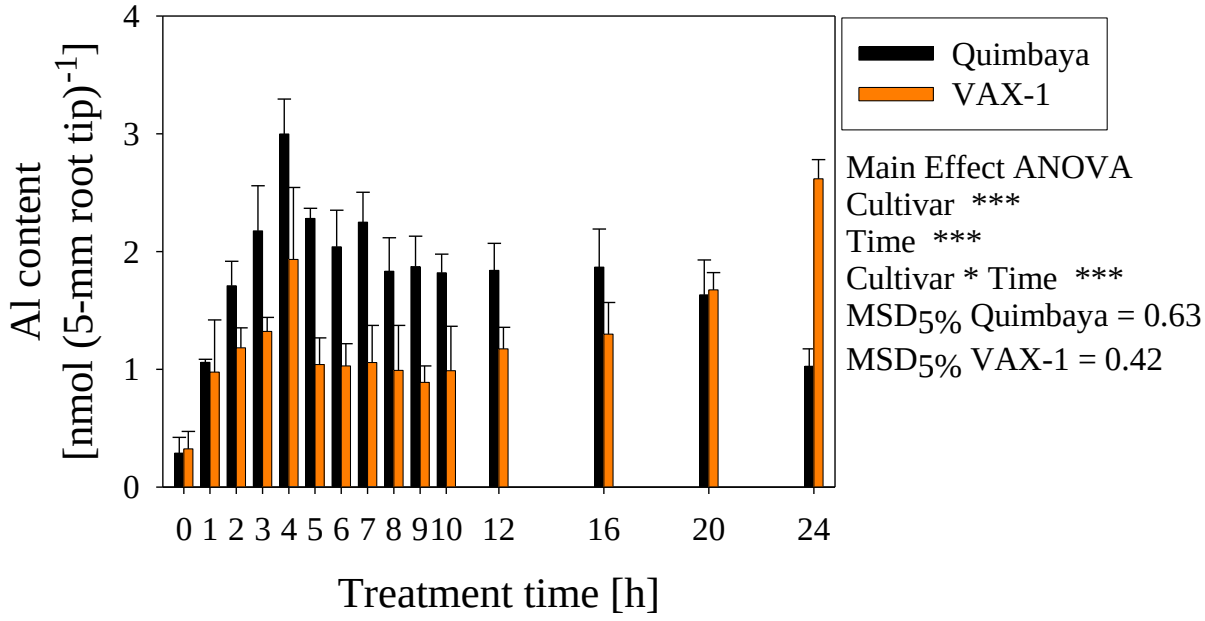


Figure 2. Al contents in 5 mm root tips of two common bean genotypes (Al-resistant Quimbaya and Al-sensitive VAX-1). Plants were grown in a solution containing 0.5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 with or without 20 µM Al for up to 24 h, pH 4.5. Bars are means ± SD of four replicates. *** Significant at $P < 0.0001$.

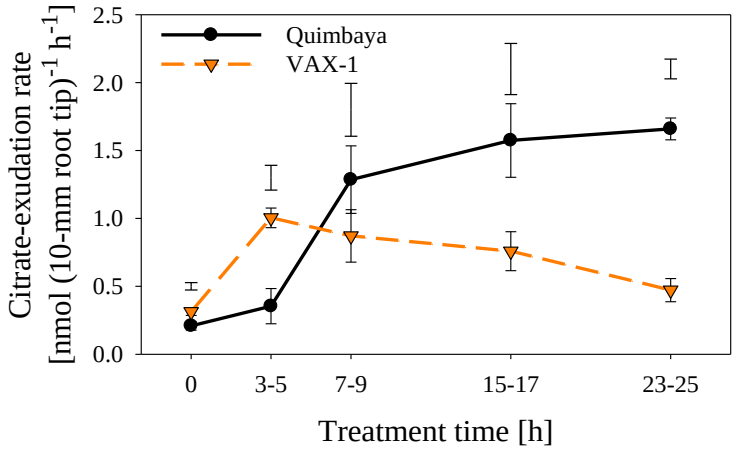


Figure 3. Dynamics of citrate exudation from root tips of two common bean genotypes (Al-resistant Quimbaya and Al-sensitive VAX-1) grown over a 25 h period (and root exudates collected and measured for 2 h intervals) in nutrient solution at 0 or 40 µM Al, pH 4.5. Bars with symbols represent the mean ± SD, n = 4. Bars on the top represent the $MSD_{0.05}$.

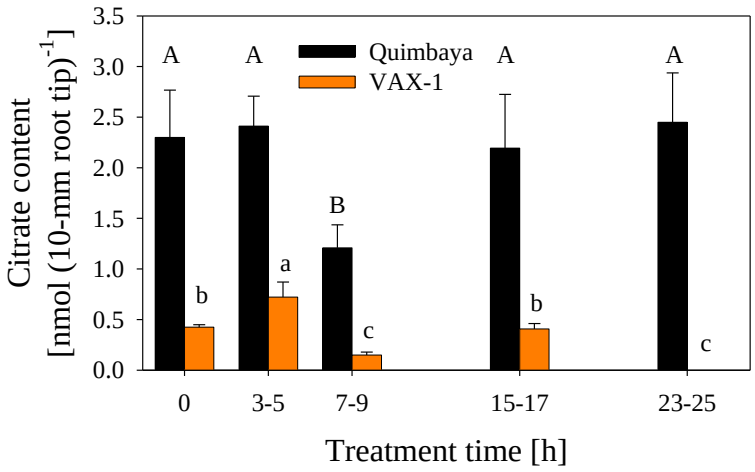


Figure 4. Effect of Al treatment on citrate content from root tips of two common bean genotypes (Al resistant Quimbaya and Al-sensitive VAX-1) grown over a 25 h period (root tips collected and measured for 2 h intervals) in nutrient solution at 0 or 40 µM Al, pH 4.5. Bars represent the mean ± SD, n = 4. Means showing similar letters are not significantly different at $p < 0.05$, Tukey-test. Capital letters: differences during the time for Quimbaya. Small letters: differences during the time for VAX-1.

Conclusions: The results shown here demonstrate that genotypic differences in Al resistance in common bean are not constitutive but build up during medium-term exposure of the roots to Al. For this acquisition of Al resistance the release of organic acid anions, particularly citrate, but even more decisively the maintenance of high internal citrate contents appear to be decisive.

3. Osmotic stress reduces Al accumulation in *Phaseolus vulgaris* L. root apex by changing cell wall porosity

Contributors: Zhong-Bao Yang, Dejene Eticha and Walter J. Horst

Rationale: Aluminium (Al) toxicity and drought are the two major abiotic stress factors limiting common bean production in the tropics. Our previous studies indicated that PEG-induced osmotic (drought) stress lead to amelioration of Al-induced inhibition of root elongation in the Al-sensitive genotype VAX 1 and greatly decrease Al accumulation in the 1-cm root apices even when the roots were physically separated from the PEG solution using dialysis membrane-tubes, which was not due to lower phyto-toxic Al concentration in the treatment solution, reduced negativity of the root apoplast, or enhanced citrate exudation. In light of this, further studies were progressed to better understand the corresponding physiological mechanism in the present study.

Materials and Methods: *Plant materials and growing conditions:* Seeds of Al-sensitive common bean genotypes VAX-1 were germinated in filter paper sandwiched between sponges. After three days, uniform seedlings were transferred to a continuously aerated simplified nutrient solution containing 5 mM $CaCl_2$, 1 mM KCl and 8 μM H_3BO_3. Plants were cultured in a growth chamber under controlled environmental conditions of a 16/8 h light/dark cycle, 27/25 ºC day/night temperature, 70% relative air humidity, and a photon flux density of 230 μmol m^{-2} s^{-1} of photosynthetically active radiation at plant height. The pH of the nutrient solution was gradually lowered to 4.5 within two days.

If not otherwise mentioned PEG 6000 (PEG) was used. In some experiments different PEG 6000 concentrations were used. The corresponding osmotic potentials (OPs) of the 0, 50, 100, 150, 200 and 250 g L^{-1} PEG 6000 solutions were 0.00, -0.06, -0.24, -0.60, -1.20 and -2.10 MPa, respectively, measured with a cryoscopic osmometer (Osmomat 030, Gonotec GmbH, Berlin, Germany).

The uptake of Al^{3+}, La^{3+}, Sr^{2+} in 1-cm root apices

To study the effect of PEG on the accumulation of La^{3+}, Sr^{2+} in the root apices, intact plants were pre-treated with the simplified nutrient solution and 0 or 50, 100, 150, 200 g L^{-1} PEG (pH 4.5) for 8 h. Then the plants were treated with 25 μM $AlCl_3$, 5 μM $LaCl_3$ or 2.5 mM $SrCl_2$ plus PEG (0 – 200 g L^{-1}) in the same nutrient solution for 1 h, pH 4.5.

Isolation of cell-wall material

After pre-treating with PEG (0 – 200 g L^{-1}) for 24 h, thirty root tips of 1-cm length were excised and transferred to 1 ml of 96% ethanol (method A) or immediately frozen in liquid nitrogen and then ground to fine powder with mortar and pestle in liquid nitrogen before 1 ml of 96% ethanol was added (method B). Cell-wall material was prepared as alcohol-insoluble residue after repeated washing with ethanol, modified after Schmohl and Horst (2000). Root samples were thoroughly homogenized in ethanol using a mixer mill at a 30 cycles s^{-1} for 2 min. The homogenization was repeated two times. Then the samples were centrifuged at 23,000 g for 15 min and the supernatant was discarded. One millilitre of 96% ethanol was added and the pellet was re-suspended. The washing procedure was repeated twice. The remaining CW material was dried using a centrifugal evaporator (RC10-22T, Jouan SA, France), weighed, and stored at 4°C for further use.

The effect of different molecular weight PEGs on root growth and Al accumulation in the root

To compare the effect of different molecular weight PEGs on root growth and Al accumulation in root apices, plants were pre-treated with PEG 1000, 3000 and 6000 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at different OPs (0, -0.06, -0.24, -0.60 MPa) for 8 h in simplified nutrient solution, pH 4.5. Then half of the plants were harvested for the determination of root elongation. The remaining plants were continued to grow for 1 h in the same solutions in the presence of 25 μM Al, pH 4.5. After the Al treatment 1-cm root tips were excised for Al analysis.

Measurement of root-elongation rate

Two hours before the treatment was initiated tap roots were marked three centimetres behind the root tip using a fine point permanent marker (Sharpie blue, Stanford) which did not affect root growth during the experimental period. Root elongation was measured after the treatment period using a mm scale.

Freeze-fracture scanning electron microscopy

After treating the plant with PEG 6000 and PEG 1000 (–0.60 MPa OP) for 4 h, root tips were excised about 7 mm from the root apex and placed onto a custom-made specimen holder, partly embedded in Tissue-Tek OCT Compound (Sakura, Fine Technical, Tokyo, Japan), and then quickly frozen with liquid nitrogen. Frozen specimens were rapidly transferred to a pre-cooled (–150°C) specimen stage in a freeze-etch unit and fractured with knife and tweezers at a distance of about 3 mm from the root apex. The samples were then etched for 15 min at 110°C under 10^{-6} Torr to remove surface ice. The structure of

root tip cross-sections was examined using a scanning electron microscope (SEM, JSM- 5600 LV, Jeol, Tokyo, Japan) after gold sputtering with high vacuum in the SEI modus at 9 kV. The sample was kept at –95°C by means of a Gatan Alto 2100 cryo system. Image recording was done with a Point Electronic, DISS5 scanning system.

Determination of Al, La, Sr

For the determination of Al, La and Sr, 1-cm root tips or cell-wall material were digested in 500 µl ultra-pure HNO_3 (65%, v/v) by overnight shaking on a rotary shaker. The digestion was completed by heating the samples in a water bath at 80°C for 20 min. Then 1.5 ml ultra-pure deionised water was added after cooling the samples in an ice-water bath. Aluminium was measured with a Unicam 939 QZ graphite furnace atomic absorption spectrophotometer (GFAAS; Analytical Technologies Inc., Cambridge, UK) at a wavelength of 308.2 nm after appropriate dilution, and an injection volume of 20 µl. La, Sr, and Rb were measured by inductively coupled plasma mass spectroscopy (ICP-MS) (7500cx, Agilent Technology, Santa Clara, California, USA) after appropriate dilution.

Results: Al accumulation in 1-cm root apices of intact plants (Figure 1A) and Al binding to the CWs of these root tips (Figure 1A′) decreased with increasing PEG concentration (0 – 150 g L^{-1}) in the treatment solution. A similar decreasing tendency was also observed for La, although the relative change was much lower compared to Al (Figures 1B, B′). Unlike that of Al and La, Sr uptake/binding was not reduced by PEG treatment (Figures 1C, C′). A higher concentration of PEG (200 g L^{-1}) did not further reduce Al and La uptake and its binding to the CW of root tips (Figure 1). A PEG supply of 250 g L^{-1} was found to be lethal to the plants since it seriously damaged the root system (data not shown).

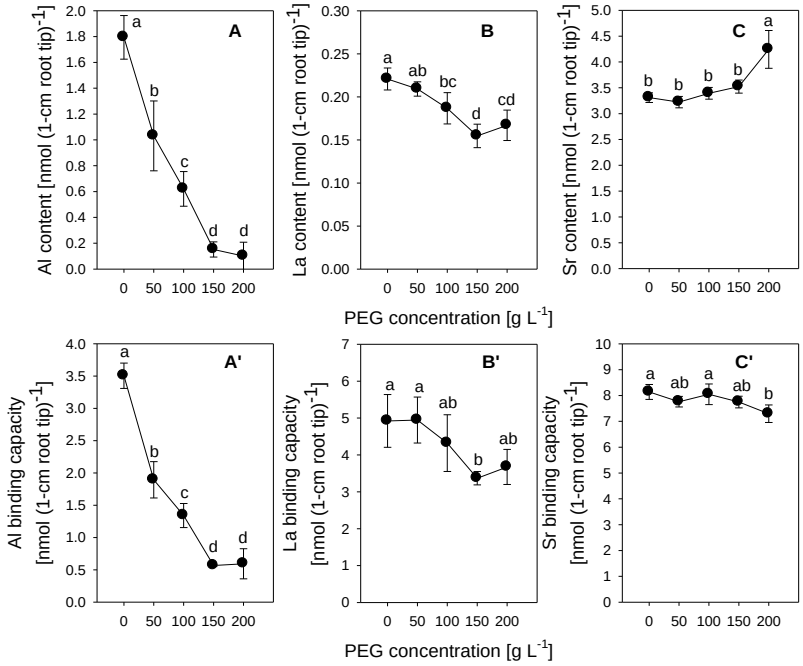


Figure 1. Effect of PEG treatment on Al, La and Sr accumulation of 1-cm root tips (A, B, C) and binding of cell-wall material isolated from 1-cm root tips (A′, B′, C′) of Al-sensitive common bean genotype (VAX 1). (A, B, C) Plants were pre-treated with PEG (0 – 200 g L^{-1}) for 8 h in a simplified solution (pH 4.5) containing 5 mM $CaCl_2$, 1 mM KCl and 8 µM H_3BO_3. Then the plants were supplied with 25 µM $AlCl_3$, 5 µM $LaCl_3$, or 2.5 mM $SrCl_2$ in the presence of PEG (0 – 200 g L^{-1}) in the same nutrient solution for 1 h as described above. (A′, B′, C′) Plants were pre-treated with PEG (0 – 200 g L^{-1}) for 24 h in the simplified solution. Then 30 root tips (1-cm) were harvested for each sample and cell-wall material isolated according to Method A described in materials and methods. Then the isolated cell-wall material was treated with 1 ml 300 µM Al, 300 µM $LaCl_3$, or 450 µM $SrCl_2$ for 30 min, pH 4.3. Bars represent means ± SD (n = 4). Means with the same letters are not significantly different at $P < 0.05$ (Tukey test).

To elaborate the role of PEG-induced alteration of cell-wall structure on Al binding, a simple physical method (method B) was used to destroy the CW structure by vigorously grinding the root apices with mortar and pestle in liquid nitrogen. PEG pre-treatment resulted in about 70% reduction of Al binding when the CW structure was widely unaltered (method A; Figure 2). But by destroying the CW structure (method B) Al binding was restored in the PEG pre-treated samples. This indicates that PEG reduces CW porosity and restricts the access of Al ions to binding sites.

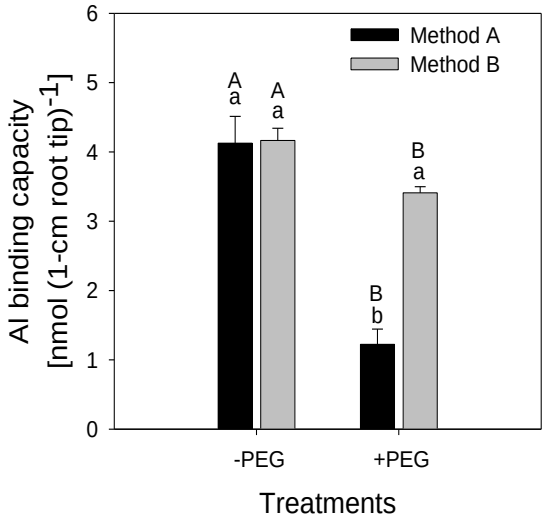


Figure 2. Al^{3+} binding of cell-wall material isolated from 1-cm root tips of Al-sensitive common bean genotype (VAX 1). Plants were pre-treated without or with 150 g L^{-1} PEG for 24 h in a simplified solution (pH 4.5) containing 5 mM $CaCl_2$, 1 mM KCl and 8 μM H_3BO_3. Then, thirty root tips (1-cm) were harvested and cell-wall material was isolated according to method A or method B, described in materials and methods. Then the isolated fine cell-wall powder was treated with 1 ml 300 μM Al for 30 min, pH 4.3. Bars represent means ± SD, n = 4. Means with the same small letter and capital letter are not significantly different at $P < 0.05$ (t test) for the comparison of the method of CW isolation within PEG pre-treatments and comparison of PEG pre-treatments within the method of CW isolation, respectively.

The effect of PEG 6000, PEG 3000, and PEG 1000 on Al contents in the root tips was compared at the same OPs corresponding to PEG 6000 concentrations of 0, 50, 100, 150 g L^{-1}. The root elongation rate was decreased with decreasing OP independent of the molecular weight of the PEG (Figure 3A). However, PEG 6000 reduced the Al contents of the root tips much more efficiently than PEG 3000 and particularly PEG 1000 (Figure 3B).

The effect of different molecular weight PEG on the root-tip structure has been studied using freeze-fracture electron microscopy. The resolution of the technique did not allow to draw any conclusion about the cell wall structure. However, the root cross-sections shown in Figure 4 clearly showed that in spite of comparable osmotic stress induced by the different molecular weight PEG (compare Figure 3) the effects on the root structure were different. In roots exposed to PEG 6000 (Figure 4C, F) the epidermis and the outer cortical cell layers were very closely packed and nearly all intercellular spaces disappeared. In contrast, PEG 1000 (Figure 4B, E) did hardly affect the intercellular space compared to the control (Figure 4A, D) indicating that in addition to osmotic stress PEG 6000 dehydrates the root apoplast more than PEG 1000.

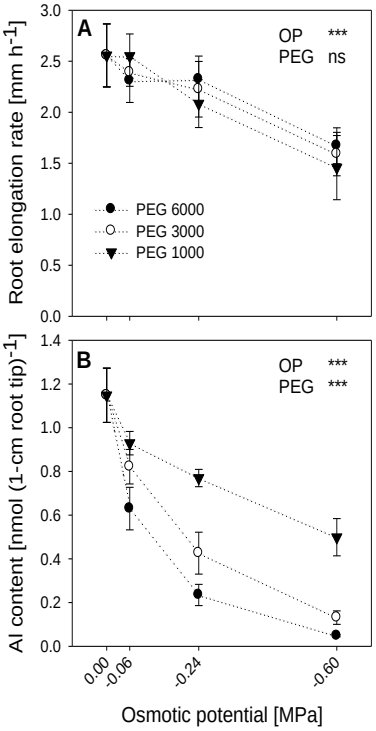


Figure 3. Effect of different molecular weight PEGs on root growth and Al accumulation in root tips of Al-sensitive common bean genotype (VAX 1). (A) Plants were pre-treated with different molecular weight PEGs at different osmotic potentials for 8 h. (B) Plants were pre-treated with different molecular weight PEGs at different osmotic potentials for 8 h, and then treated with 25 μM Al for 1 h in the presence of different molecular weight PEGs for 1 h. The background solution of the above treatment solution was the simplified solution containing 5 mM $CaCl_2$, 1 mM KCl, and 8 μM H_3BO_3, pH 4.5. Bars represent means ± SD, n = 4. For the ANOVA, *** denote significant differences at $P < 0.001$; ns = not significant (F test).

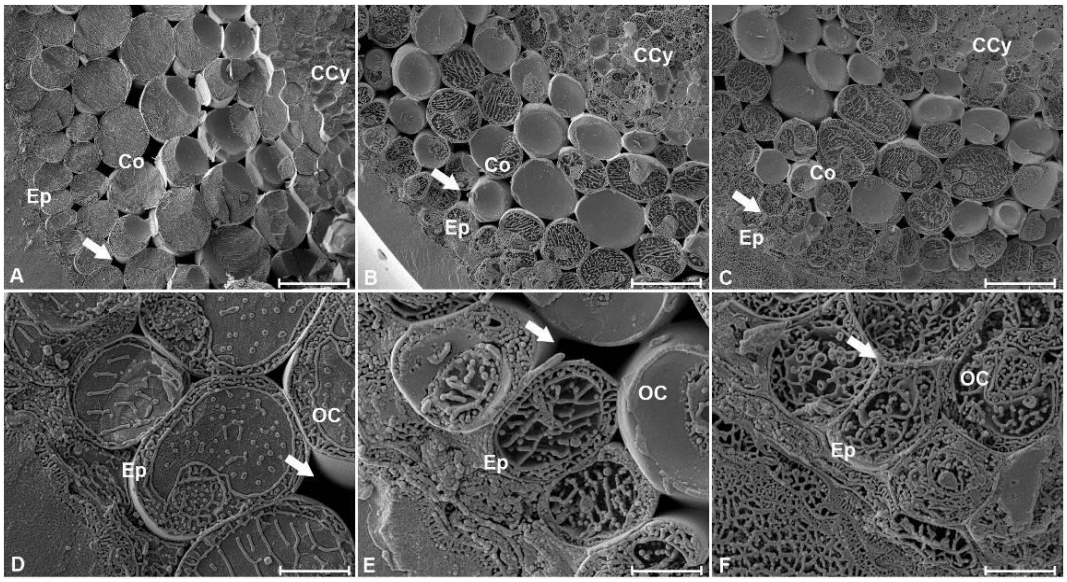


Figure 4. Freeze-fracture scanning electron micrographs of root-tip cross-sections (1-5 mm from the root apex) of common bean genotype VAX 1 grown for 4 h in the presence of different molecular weight PEG. Upper row show root cross-section segments from the epidermis (Ep) through the cortex (Co) to the central cylinder (CCy), lower row pictures show the epidermis and one outer cortical cell layer (OC). Arrows indicate the presence (A, B, D, E,) or absence (C, F) of intercellular spaces between the epidermis and the outer cortical cell layer. A, D: control; B, E: PEG 1000; C, F: PEG 6000. Scale bars correspond to 40 μm in A, B and C, 10 μm in D, E and F.

Discussion and Conclusions

In comparison with La, Sr, and Rb, the strong reduction of cation accumulation in the root apex by osmotic stress appears to be specific to Al (Figure 1). The specificity of cation accumulation might be related to the hydrated ionic radius of the cations: Al^{3+} (0.475 nm) > La^{3+} (0.452 nm) > Sr^{2+} (0.412 nm) = Ca^{2+} (0.412 nm) > K^{+} (0.331 nm) > Rb^{+} (0.329 nm). Since the pore size of the CW plays an important role in apoplastic transport of water, ions, metabolites and proteins, the differences between the ions in Al accumulation of the PEG-exposed root apices may suggest that PEG (osmotic stress) affects CW porosity. This assumption is supported by the fact that a similar reduction in accumulation specific for Al could also be observed in cell walls isolated from PEG-treated root tips (Figure 1). Microscopic evaluation showed that the CW material was fairly intact (not shown) indicating that the CW porosity was not disrupted. After physically destroying the structure of the CW, Al binding to the CW was almost restored (Figure 2).

Generally, the pore diameter of the plant CW is in the range of 3.5 - 5.5 nm, which mainly depends on CW structure, hydrophobicity, CW chemical composition and physical properties. Thus any change of these factors may result in subsequent alteration of porosity. Water is the most abundant component of the CW making up about two thirds of the wall mass in growing tissues. This water is located mainly in the matrix (≈ 75 – 80% water), which suggests that the matrix has properties of a relatively dense hydro-gel. This visco-elastic nature of the plant CW allows it to respond to stresses and limitations imposed upon it. Loss of water from the wall matrix can result in serious disruption to polymer organization. One obvious effect is that polymers usually well separated in the hydrated wall are brought in close proximity to each other, thus causing polymer adhesion or cross-linking under water stress.

The extent of loss of water from the apoplast and consequently shrinkage of the root structure appeared to be dependent of the molecular size of the applied PEG: PEG 6000 > PEG 3000 >> PEG 1000 (Figure 4). The difference between the PEG sources at the same OP of -0.60 MPa might be related to the penetration of the PEG molecules into the root apoplast: the higher the hydrodynamic radius the better the exclusion from the apoplast and consequently the dehydration of the apoplast. The estimated hydrodynamic radii of PEG 6000, 3000, and 1000 are 2.7, 1.6, and 0.89 nm, respectively.

Also, from our previous results, the rapid recovery of Al accumulation in the living root apex after transfer of the roots into PEG-free solution suggests that the water content of the apoplast is a decisive factor for PEG-induced alteration of CW porosity. However, since the restoration of the Al accumulation capacity of the cell walls after the cessation of the PEG stress could only be observed in living root apices but not in ethanol-insoluble CW material isolated from root apices pre-treated with PEG, it is speculated that some proteins related to the modification of the CW structure are involved in the PEG 6000 (osmotic stress)-induced alteration of CW porosity. This needs to be substantiated through further physiological and molecular studies.

In conclusion, the observed results provide circumstantial evidence that the osmotic stress-inhibited Al accumulation in root apices and thus reduced Al-induced inhibition of root elongation in the Al-sensitive common bean genotype VAX 1 is related to the alteration of CW porosity resulting from PEG 6000-induced dehydration of the root apoplast.

4. Analysis of gene expression in response to Al treatment in common bean (*Phaseolus vulgaris*) genotypes

Contributors: D. Eticha, M. Zahn, M. Bremer, Z. Yang, A.F. Rangel, I. M. Rao and W. J. Horst

Rationale: Common bean (*Phaseolus vulgaris* L.) is produced in the tropics by small scale farms where unfavourable edaphic factors limit the yield potential. Among others, soil acidity which covers about 40% of the world arable land (Von Uexküll and Mutert, 1995) accounts for 30 – 40% yield reduction in Africa and Latin America (CIAT, 1992). The crop yield on acid soils is mainly limited by aluminium (Al) toxicity. Comparing two contrasting bean cultivars Quimbaya (Al-resistant) and VAX 1 (Al-sensitive), in our previous studies, we have found that Al resistance in common bean is attributed to the release of citrate by the root apex. Organic acid anions such as citrate, malate and oxalate detoxify Al through forming a non-phytotoxic organic acid-Al complex. There are two patterns of organic acid secretion: pattern I plants release organic anions immediately after the onset of Al treatment while in pattern II plants, organic anion release starts after a lag phase of several hours. This suggests that in pattern I the organic anion release mechanism is constitutively expressed, whereas in pattern II plants the induction of the resistance mechanism involves gene expression, and new protein synthesis. Common bean is proved to be a typical

pattern II plant. The delay in citrate exudation was not due to the limitation of internal citrate reserve but to the absence of citrate permeases. Aluminium resistance genes of several plant species have been identified and found to encode membrane proteins which mediate the exudation of organic acid anions from the root. These proteins belong to two families, *ALMT* and *MATE*. The *ALMT* (Al-activated malate transporter) facilitates malate efflux in plant species that depend on malate exudation as Al resistance mechanism. On the other hand, the *MATE* (multidrug and toxin extrusion) proteins are citrate transporters which play a decisive role in Al-induced citrate exudation.

Based on our previous observations it was hypothesised that the expression of a citrate transporter and the enhanced synthesis of citrate are crucial for sustained Al resistance in common bean. Thus, the objective of this work was to analyse the expression of selected candidate genes which may have significant roles in Al resistance using quantitative real-time PCR.

Materials and Methods: *Plant material and growth condition:* Two common bean genotypes with known differential Al resistance, Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) were used in this study. The seeds were germinated in filter papers sandwiched between sponges soaked with tap water. After 4 days, uniform seedlings were transferred to 18-liter pots with a continuously aerated nutrient solution (containing 5 mM $CaCl_2$, 1 mM KCl and 8 µM H_3BO_3) in a controlled climate chamber, with a 16/8 h light/dark regime, 27/25 °C day/night temperatures, 70% relative air humidity, and a photon flux density of 230 µmol m^{-2} s^{-1} (photosynthetic active radiation) at the plant canopy. The pH of the nutrient solution was gradually lowered to 4.5 within two days. Then the plants were treated without or with 20 µM $AlCl_3$ for various durations of time up to 24 hours. Root growth was measured at 4, 8 and 24 h of Al treatment.

Determination of citrate exudation and citrate contents of root apices: Citrate exudatation from root tips and citrate contents of root tips were determined as described by Rangel et al., 2010. Briefly, plants were pre-treated without or with 20 µM Al for 3, 7, and 23 h at pH 4.5 as described above. To collect root exudates from intact root apices, 12 pre-treated plants were bundled in filter paper soaked with nutrient solution. Approximately, 1 cm of the main root apex of each plant was immersed into 18 ml of a constantly aerated collection solution containing 5 mM $CaCl_2$, 8 µM H_3BO_3 and 0 or 40 µM $AlCl_3$, pH 4.5, in 20-ml poly prep filtration columns (BioRad Laboratories, Richmond, CA).The Al concentration in the collection medium was doubled in order to compensate for the small volume and thus low total Al supply. After 2 h of incubation, the collection solution containing the root exudates was immediately frozen at −20°C for later citrate determination. At the end of incubation period, the root tips (1 cm) were excised with a razor blade, rinsed with double deionized water, transferred to Eppendorf reaction vials and fixed immediately in liquid nitrogen to measure the citrate content in the root tissue. The citrate concentrations in the root exudates as well as in the root tissue extracts were measured by isocratic high pressure liquid chromatography (HPLC, Kroma System 3000, Kontron Instruments, Munich, Germany).

Candidate-gene selection and primer design: Candidate genes were selected either from our own SSH library or from public data base based on our previous results on the physiological characterisation of the mechanisms of Al resistance in common bean. The sequences of candidate genes were initially obtained from the Arabidopsis database (TAIR). Then, similar sequences of known genes of legumes and Expressed Sequence Tags (ESTs) of common bean (*P. vulgaris*) were searched and gathered for sequence alignment. Finally, primers were designed in such a way that they anneal to part of the sequence which is well conserved among the legume species. Primers were designed using Primer3 software. The list of candidate genes and their respective primer pairs are shown in Table 1.

The citrate transporter gene *MATE* is a member of a large gene family. Several ESTs of *P. vulgaris* which have similarity with known *MATE* genes were gathered and aligned to assess their homology. Based on the alignment result (Figure 1) they were grouped into 3 classes (*MATE*-a, *MATE*-b, and *MATE*-c) and appropriate primers were designed as described above. *MATE*-a and *MATE*-c have nucleotide-sequence similarities of 81% and 75%, respectively, with the Arabidopsis *MATE* gene (Locus: AT1G51340). Likewise, *MATE*-b has 72% similarity with the Arabidopsis *FRD3* (ferric reductase defective 3) gene (Locus: AT3G08040).

Table 1. List of genes and specific primer pairs used for quantitative gene expression analysis.

Candidate gene	Primer pairs (5' → 3')*	Amplicon size (bp)	GenBank Acc. #
PFK (Phosphofructokinase)	(+) ACCCTTGCAAGTCGAGATGT (-) CTGCACACTCTCGGAAACAA	171	FE688067
PEPC (Phosphoenolpyruvate carboxylase)	(+) TGGCTCCTTCCAAAGTGAGT (-) TCCTCCCCGTGTAAATTCTG	100	AF288382
CS (Citrate synthase)	(+) CGACGGATATTCAAGGATGG (-)CGTGATCACTGTGGATGGAA	142	FE693132
Aco (Aconitase)	(+) TCCAGTGTGTTGCCTGACAT (-) GACTTGGGGTCCCATGAGTA	116	CV536664
ICDH (Isocitrate dehydrogenase)	(+) GCTCATTTTGCCTTTCCTG (-) TTCACACGAGCTTCATCTGG	165	SSH library
MDH (Malate dehydrogenase)	(+) CCAACTGCAAGGTTCTGGTT (-) GCCCTGTTGTGATCCAATCT	124	FE677903
STOP1 (Transcription factor)	(+) GCTCTAACTGCCGATGAGAA (-) TCTCTCCAGCTCCTCCTGAA	159	SSH library
MATE-a (Citrate transporter family)	(+) GCTGGATGCAGTTTCAAGAGAG (-) ACTCCAGCAGCTGCAAGTTC	138	CV535133
MATE-b (Citrate transporter family)	(+) TGCTGTTCAAGCCATTCTAGC (-) TCCAACAGCAAGAGAGAGTCC	124	CV534527
MATE-c (Citrate transporter family)	(+) GTGACACTGGCTGCATCATT (-) GAGAAACTGCCAACCAAACC	91	FD792891
ALS3 (Al-sensitive3)	(+) ACAAGCTTGGCCTCCAGATA (-) GCGTTGTCCTGGTTGAAGAT	106	CV532021
ALMT1 (Al-activated malate transporter)	(+) TTCGCCCCATCTGGGCTGGT (-) TCCGGGGTTTCACTGCCATCA	118	CV543751
VDAC (Voltage dependent anion channel)	(+) TGCCTCGTTGACTCTGAATG (-) CCGAGGTACCAAGGATGTGA	146	SSH library
β-tubulin	(+) CCGTTGTGGAGCCTTACAAT (-) GCTTGAGGGTCCTGAAACAA	117	CV530631

* (+) and (-) indicate forward and reverse primers, respectively

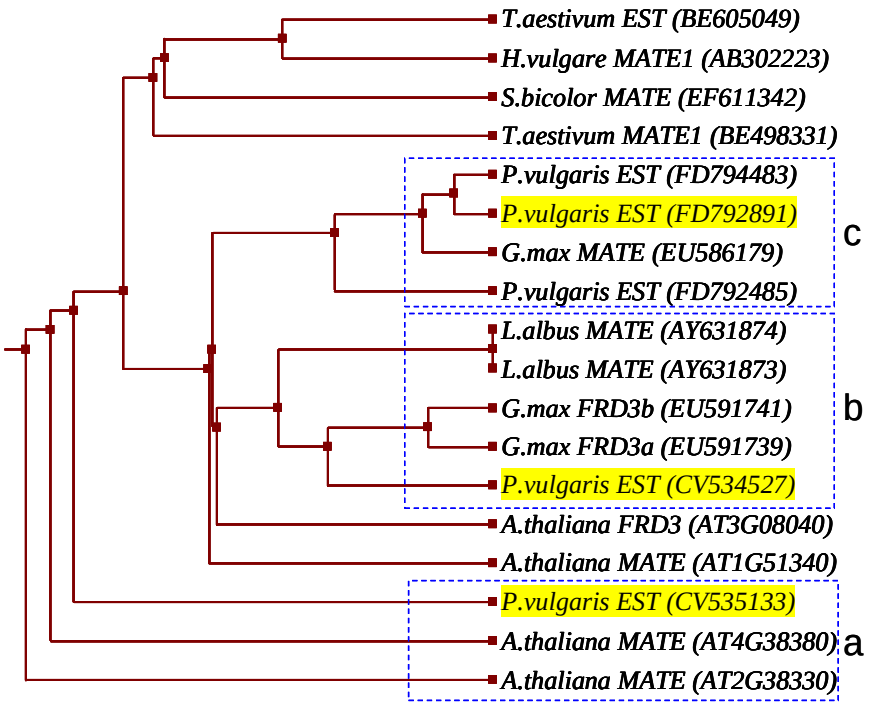


Figure 1. Sequence alignment tree of *MATE* genes along with *Phaseolus vulgaris* ESTs having high similarity to the known *MATE* genes. The alignment was done using online MAFFT software.

Quantitative real-time PCR: Two common bean genotypes Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) were grown and treated without or with 20 µM $AlCl_3$ for 1, 2, 3, 4, 8 and 24 h as described above. At the end of each treatment time, roots were rinsed with distilled water and 10 root tips (1 cm) per plant were harvested and shock-frozen in liquid nitrogen. Root tips of 15 plants per treatment were bulked and ground to powder in liquid nitrogen. Total RNA was isolated from the root tips as described above. First strand cDNA was synthesised using RevertAid H-Minus First Strand cDNA Synthesis Kit (Fermentas, www.fermentas.com). Random hexamer primers were used for this purpose. The reaction was stopped by heating at 70°C for 10 min followed by 20 min incubation at 37°C after addition of 10 U RNAse-H (EPICENTRE Biotechnologies, www.epibio.com). Quantitative real-time PCR (qRT-PCR) was undertaken using the Applied Biosystems StepOne Plus thermo-cycler (www.appliedbiosystems.com). The SYBR Green detection system was used with self-prepared SYBR Green master mix including a passive reference dye, ROX. The constituents of the qRT-PCR reaction mix were 1x Hot Start PCR Buffer, 3.6 mM $MgCl_2$, 0.5 µM ROX, 0.1x SYBR Green-I, 200 µM each dNTPs (dATP, dTTP dCTP dGTP), 252 nM each forward and reverse primers, 0.75 U Hot-Start Taq DNA Polymerase, 2 ng/µl cDNA template and Ultra pure DNAse/RNAse-free distilled water in a final volume of 25 µl. The qRT-PCR cycling stages consist of initial denaturation at 95°C (3 min), followed by 45 cycles of 95°C (15 sec), 60°C (30 sec), 72°C (30 sec), and a final melting curve stage of 95°C (15 sec), 60°C (1 min) and 95°C (15 sec). The fluorescence signal was recorded during the strand elongation step at 72°C and the melting curve stage at every 0.3°C temperature ramp. Samples for qRT-PCR were run in three biological replicates and two technical replicates. Relative gene expression was calculated using the comparative $\Delta\Delta C_T$ method according to Livak and Schmittgen (2001). For the normalization of gene expression, three housekeeping genes, namely 18S rRNA, actin, and β-tubulin were tested and the latter was found to be more stable. Accordingly, β-tubulin was used as an internal standard and the control plants of the Al-resistant genotype Quimbaya were used as reference sample. The PCR efficiencies of the β-tubulin and the target genes were comparable and thus relative gene expression was calculated without efficiency correction.

Results: Root growth of both genotypes Quimbaya and VAX 1 was reduced by more than 50% within four hours of Al treatment (Figure 2A). However, Quimbaya gradually recovered from the initial Al stress and recovered to nearly full growth within 24 h. In contrast, VAX 1 showed a transient recovery after eight hours, but later the growth was again severely inhibited. Citrate exudation from root tips was determined

after 4, 8 and 24 h Al treatment (Figure 2B). Citrate exudation was induced by Al treatment after a lag phase of 4 h in genotype VAX 1, but in genotype Quimbaya the lag phase lasted more than 4 h. After 8 h the exudation rate remained constant in genotype VAX-1, but in genotype Quimbaya the citrate exudation-rate steeply increase up to 24 h of Al treatment. The Al-induced exudation of citrate might be related to the citrate contents of the root-tip tissue. Therefore, the citrate contents of the 1 cm root tips were determined after the collection of root exudates in order to study the effect of Al-treatment duration on the dynamics of the citrate content in the root tissue (Figure 2C). Genotype Quimbaya had constitutively higher citrate contents than VAX 1. After 8 h Al treatment the citrate content of both genotypes were significantly reduced compared to their respective controls. This was related to the enhanced citrate exudation (Figure 2B). Striking differences between the two genotypes were observed in the ability to restore the tissue citrate content. While the Al-resistant genotype Quimbaya was able to restore its tissue citrate level, in the Al-sensitive genotype VAX 1 the citrate pool was depleted after 24 h. A close relationship existed between the dynamics of citrate exudation after the lag phase (Figure 2B) and the recovery of root growth after 8 to 24 h Al treatment (Figure 2A).

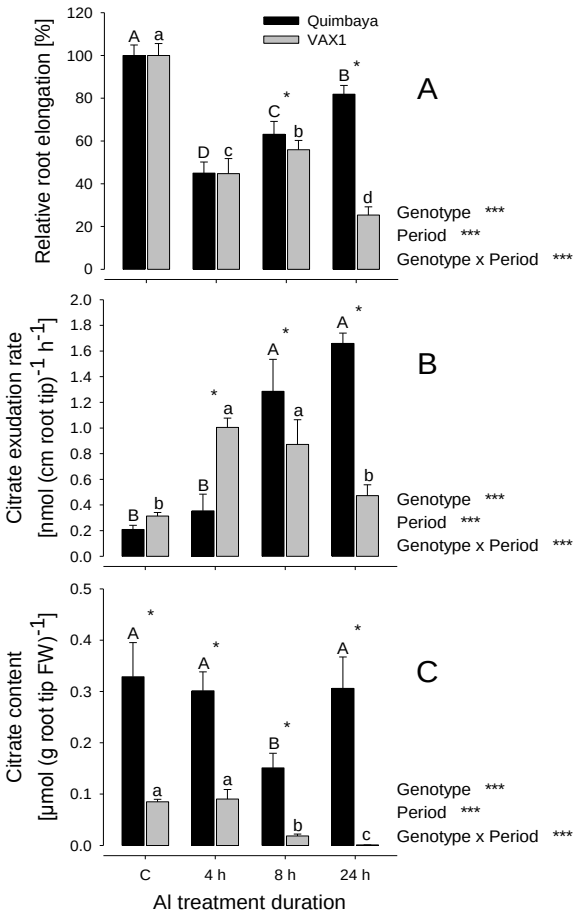


Figure 2. (A) Root growth, (B) citrate exudation rate, (C) and citrate content of 1 cm root tips of two common bean genotypes Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) grown in simplified nutrient solution treated without or with 20 µM Al, pH 4.5 for up to 24 h. For the determination of citrate exudation and root-tissue citrate-content, plants were pre-cultured in nutrient solution without or with 20 µM Al, pH 4.5 for 3, 7, or 23 h. Root exudates were collected for a period of 2 h in simplified collection solution without or with 40 µM Al, pH 4.5. Citrate content of the root tissue was determined at the end of collection period. Bars are means +SD of four to six replicates. For the analysis of variance, *** denote significance at $P < 0.001$. Means with the same letter are not significantly different between exudation periods for Quimbaya (capital) and VAX-1 (small); * on top of data points show significant differences between genotypes within each treatment time (Tukey test, $P < 0.05$). The letter 'C' on the X-axis stands for control plants not treated with Al.

Candidate genes were selected based on the physiological mechanisms of Al resistance in bean, which is a sustained release of citrate from the root tips (Figure 2). The expression of two groups of genes was closely investigated using qRT-PCR. The first group includes genes encoding the enzymes involved in citrate metabolism, while the second group consists of genes encoding ion transporters. Aluminium treatment did not significantly alter the expression of genes coding for enzymes which play role in citrate synthesis and degradation (Figure 3). Besides, there were little or no genotypic differences in Al-induced changes in gene expression. However, constitutively lower expression of genes coding for PEPC and ICDH was observed in VAX 1 than in Quimbaya. The expression pattern did not change when the plants were treated for 8 or 24 hours (data not shown).

The release of organic acid anions is mediated by membrane-localized anion transporters, whose expressions were known to be regulated by the *STOP1* transcription factor. In beans, short-term Al treatment (4 h) slightly increased the expression of *STOP1* (Figure 4) but the expression levels of *MATE*-a, *MATE*-b and *ALS3* were greatly enhanced by Al treatment in both bean genotypes. Yet, Al did not affect the transcript abundance of *MATE*-c, *ALMT1*, and *VDAC* (Figure 4). In order to find out the exact timing of *MATE*-gene induction, root samples were collected at one hour interval during the first 4 h of Al treatment. Enhanced expression of the *MATE*-a gene was observed as early as 2 h Al treatment in VAX 1, but in Quimbaya the expression was delayed until 3 h (Figure 5). The expression levels continued to increase with time in both genotypes. The expression level was much higher in VAX 1 than in Quimbaya. The earlier induction of *MATE* genes in this genotype is in agreement with the earlier induction of citrate exudation in VAX 1 (Figure 2B).

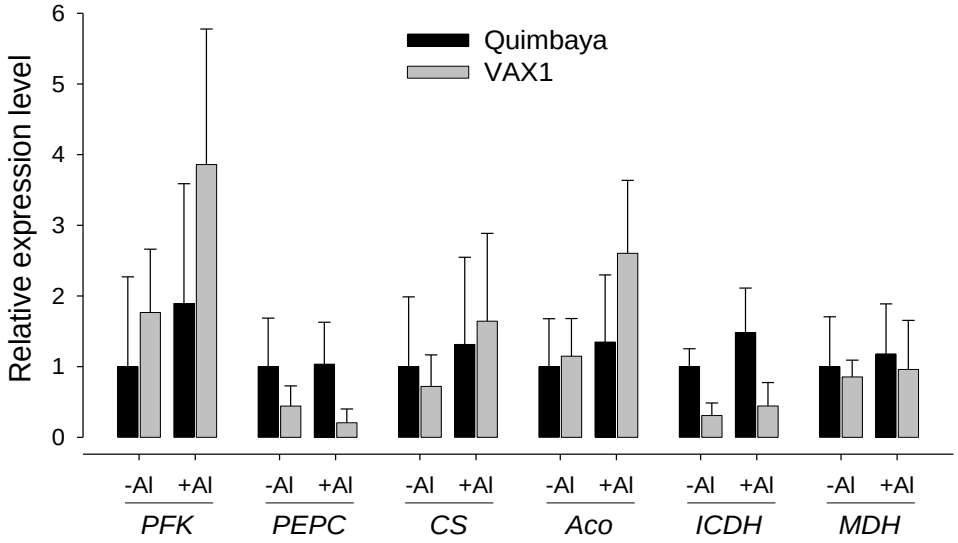


Figure 3. Expression of genes encoding enzymes involved in citrate metabolism in common bean genotypes Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) grown in nutrient solution treated without or with 20 µM Al for 4 h. Total RNA was extracted from root tips. Quantitative RT-PCR was performed using the β-tubulin gene as internal standard, and untreated plants of the Al-resistant genotype Quimbaya as calibrator. Relative gene expression was calculated from three biological and two technical replicates.

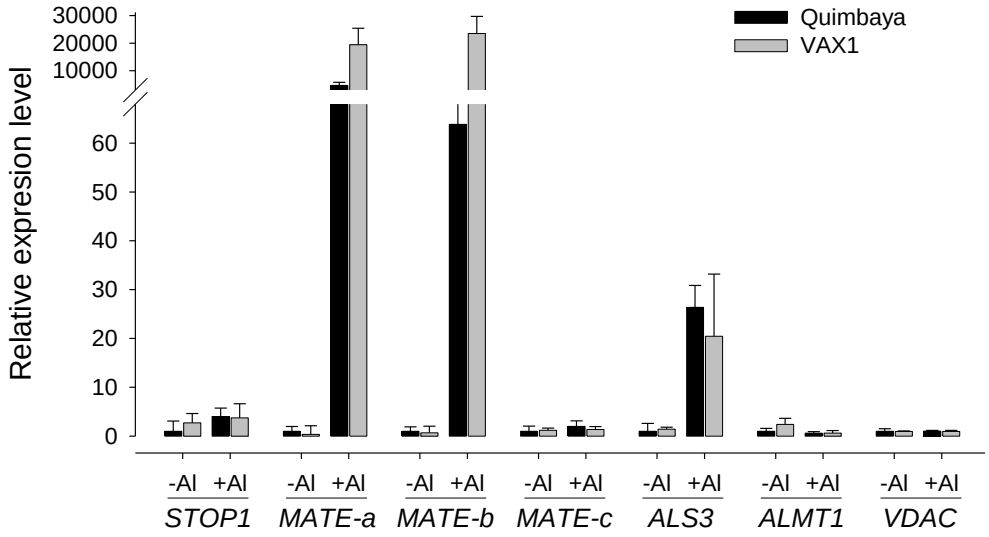


Figure 4. Expression of genes regulating/encoding ion transporters in the common bean genotypes Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) grown in nutrient solution treated without or with 20 µM Al for 4 h. Total RNA was extracted from root tips. Quantitative RT-PCR was performed using the β-tubulin gene as internal standard and untreated plants of the Al-resistant genotype Quimbaya as calibrator. Relative gene expression was calculated from three biological and two technical replicates.

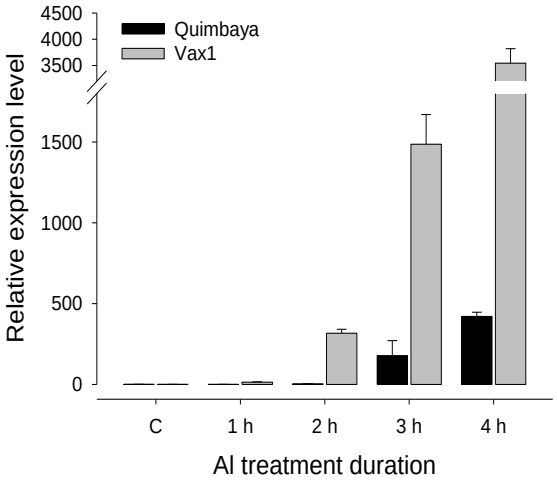


Figure 5. Expression of *MATE-a* genes under short term Al treatment in the common bean genotypes Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) grown in nutrient solution treated without or with 20 µM Al for up to 4 h. Total RNA was extracted from root tips. Quantitative RT-PCR was performed using the β-tubulin gene as internal standard and untreated plants of the Al-resistant genotype Quimbaya as calibrator. Relative gene expression was calculated from three biological and two technical replicates. The letter 'C' on the X-axis stands for control plants not treated with Al.

Discussion: The best understood mechanism of Al resistance in plants is the release of organic acid anions such as citrate, malate, and oxalate, which chelate Al and form non-toxic complexes. We observed that Al-activated exudation of citrate plays a major role in Al resistance of common bean. Citrate exudation started after about four hours of Al treatment despite the abundant citrate content in the root tissue (Figure 2B and C). Moreover, the root growth of both Al-resistant and Al-sensitive bean genotypes was equally inhibited during this lag period indicating that Al resistance in common bean is not constitutively expressed (Figure 2A). This indicates that Al resistance in bean is an inducible trait. The lag phase between the beginning of Al treatment and the onset of citrate exudation shows that the induction

process involves gene transcription and *de novo* synthesis of proteins which are necessary for citrate transport.

In the present study, the expression of genes encoding organic anion transporters was examined. Among the candidate genes tested *MATE*-a (GenBank Acc. # CV535133) and *MATE*-b (GenBank Acc. # CV534527) were strongly expressed upon Al treatment in bean. Both expressed sequence tags (denoted as *MATE*-a and *MATE*-b) of common bean have high sequence similarity to previously characterised *MATE* genes of *Lupinus albus* (GenBank Acc. # AY631874) and *Glycine max* (GenBank Acc. # EU591739 and EU591741). Nucleotide sequences of *MATE*-a and *MATE*-b have no significant similarity and also they do not belong to the same contig assembly of ESTs in the TIGR data base. Whether they are two different genes or just different sequence regions of the same gene needs to be clarified through full length cDNA sequencing.

The MATE proteins are a large family of membrane transport-proteins which have 58 members (paralogues) known just in the Arabidopsis genome. The Arabidopsis *FRD3* gene which is important for iron transport in the xylem as ferric citrate is also a MATE protein. The role of a MATE protein for Al resistance was observed in sorghum, barley and wheat. The MATE protein was described as an Al-activated citrate transporter which is responsible for Al resistance of both sorghum and barley. In sorghum the *SbMATE* was expressed only in the root tips of the Al-resistant genotype in an Al-inducible way. Similarly, barley *HvMATE* was constitutively expressed mainly in the root apices and correlated with Al-activated citrate exudation and Al resistance in a set of barley cultivars. In contrast, the *MATE* gene of bean is highly expressed in both resistant and sensitive genotypes used in the present study (Figures 4 and 5). This result corroborates the observation that citrate exudation was induced by Al in both Al-resistant and sensitive genotypes (Figure 2B). Regardless of the ample amount of citrate in the root tissue (Figure 2B), exudation started only after about 4 h of Al treatment, the time lag which is required for activation of *MATE*-gene transcription, translation and formation of the functional protein (Figure 5). After the MATE protein is in place, citrate exudation progressed and resulted in a reduction of the citrate content in the root tissue (Figure 2B and C). As a result of citrate exudation, both bean genotypes transiently recovered from the stress which equally affected both of them at the early hours of the Al treatment (Figure 2A). The remarkable difference between the two bean genotypes was observed in their capacity to replenish the tissue citrate reserve and to sustain citrate exudation in order to protect the growing root tip. The Al-resistant genotype Quimbaya was able to restore the citrate pool in the root tissue and to continue to release citrate, whereas the Al-sensitive genotype VAX 1 was unable to restore the internal citrate pool and failed to further release citrate after the short recovery period (Figure 2B and C). These observations underline that sustained synthesis of citrate as well as constant expression and activity of a citrate transporter are vital for Al resistance in common bean.

Although the role of organic acid anion exudation for Al resistance and the importance of organic acid anion transporters are currently well defined, the significance of organic acid metabolism and accumulation in the root tissue are still not well understood. In plant species, where organic acid anion release started directly after Al treatment, no correlations were observed between internal organic acid concentrations and efflux. For example, Al-sensitive and Al-resistant wheat genotypes did not differ in root concentrations of malate, although the Al-resistant genotypes released up to 10-fold more malate than the Al-sensitive genotypes. Similarly, contrasting maize genotypes did not differ in tissue citrate content and Al equally increased citrate accumulation in the root tissue of both genotypes but significant citrate exudation was only observed in the Al-resistant genotype. In contrast, in soybean (pattern II plant) the Al-enhanced internal accumulation of citrate contributed to the enhanced citrate exudation. Reports on the role of enzymes involved in the organic acid metabolism for Al-induced organic acid anion efflux are also diverse. In wheat, Al-induced malate exudation occurred without significant changes to the activities of phosphoenolpyruvate carboxylase (PEPC) or malate dehydrogenase (NAD-MDH). Moreover, the activities of these enzymes were not significantly different between genotypes. In contrast, an increased citrate synthase (CS) activity was reported in *P. vulgaris* and *Cassia tara* after Al treatment. Similarly, Al treatment enhanced the gene expression as well as enzyme activity of mitochondrial CS in soybean. Furthermore, over-expression of enzymes involved in organic acid metabolism has been proven to be effective in enhancing exudation of organic acid anions leading to Al resistance in transgenic plants of Arabidopsis, alfalfa and canola.

In our previous works, we studied the changes in activities of enzymes involved in citrate metabolism. Al treatment reduced the activity of isocitrate dehydrogenase (ICDH) leading to reduced internal citrate consumption and enhanced exudation. The citrate content in the root tissue is a function of citrate

synthesis, exudation, degradation or consumption for other metabolic functions. Accordingly, continuous release of citrate while maintaining normal citrate concentration in root tissue requires enhanced synthesis and/or reduced degradation of citrate. Reduction in cytosolic NADP-isocitrate dehydrogenase activity resulted in citrate accumulation and subsequent release from mutant carrot cells which were able to grow on insoluble phosphate sources. But our results indicate that not only the reduction of NADP-ICDH but also maintaining the activities of citrate synthase (CS) and phosphoenolpyruvate carboxylase (PEPC) are important for sustained exudation of citrate in common bean. Failure of continuous citrate exudation in the Al-sensitive bean genotype VAX 1 was mainly attributed to the constitutively lower CS activity which was further inhibited by extended duration of Al treatment. In the current study, no significant change was observed in the expression of genes encoding enzymes involved in citrate metabolism (Figure 3). Similarly, a large-scale, transcriptomic analysis of root responses to Al, using a microarray representing about 93% of the predicted genes of the Arabidopsis genome did not show any significant increase in transcript abundance for any of the 52 genes of the TCA cycle present in the micro array, except for MDH. However this does not mean that there is no change in the activity of enzymes involve in the TCA cycle. Since we clearly demonstrated the changes in the enzyme activity of the above bean genotypes we may conclude that the activities of these enzymes are regulated at the post translational level.

The role of ATP-binding cassette (ABC) transporter family proteins, *ALS1* and *ALS3* for Al resistance was observed in Arabidopsis. Plant ABC transporters that have been functionally characterised so far were known to detoxify organic and inorganic compounds by sequestering in the vacuole. Arabidopsis *als1-1* and *als3-1* mutants were hypersensitive to Al but the exact functions and substrates of ALS1 and ALS3 are not known. Whereas ALS1 is located in the tonoplast and the gene is expressed in root apices and the vascular system, ALS3 is primarily located in the plasma membrane of leaf hydathode cells, the phloem and the root cortex. The expression of *ALS3* is induced by Al and was suggested to function in channelling accumulated Al away from Al-sensitive tissues in order to protect the growing root from Al toxicity. We observed that Al treatment induced the expression of the *ALS3* gene in both in Al-resistant and sensitive bean genotypes (Figure 4). However, the suggested function of *ALS3* could not be confirmed since the sensitive cultivar continued to accumulate Al in the root tissue regardless of *ALS3* expression.

Conclusions: This study strongly suggests that *MATE* gene is responsible for Al-induced citrate exudation in common bean. The expression of this gene is a prerequisite for Al resistance. However, sustained citrate release and genotypic Al resistance additionally requires the continuous synthesis and maintenance of a cytosolic citrate pool in the root apex.

5. Improving phenotyping capacity to evaluate for aluminum resistance

Contributors: A.F. Rangel, J. Ricaurte, S. Beebe, I. M. Rao and W. Horst

Rationale: Screening for Al resistance using hydroponic-based methodology normally requires that large amounts of genotypes or accessions should be tested at the same time under the same climatic and experimental conditions. Independently of the amount of materials to be tested, obtaining healthy and homogenous plantlets or seedlings to reduce the variability originating in the germination process is of great importance. Previously, germination of bean seedlings involved use of growing mediums such as peat, sand or a mix of both substrates. However, this method is time consuming, special skills are required to clean the peat from the roots in order to reduce the interference with the Al in the nutrient solution and in many cases the roots are not uniform. Normally, it was required up to 16 h to establish, germinate and transplant a trial with 60 genotypes. In spite of careful manipulation, in the end high standard deviations did not allow clear differentiation between genotypes for their level of Al resistance.

Materials and Methods: After reviewing the previous method used in the screening of bean for Al resistance, some key modifications were made to increase the capacity to screen larger number of genotypes while reducing the time needed for the establishment and the sources of variability between replicates. These key modifications in order of importance were:

1. Replacing the sand-based germination by filter-paper/styrofoam sandwiches;
2. Manipulation of plantlets during the transplant to nutrient solution to avoid damage of basal roots (very important in the determination of root architecture parameters);
3. Measuring root length after 24 or 48 h of Al treatment using marked roots (important to correlate with physiological studies);
4. Controlling the pH of the nutrient solution, especially during the preparation of the Al treatments;

5. Controlling the aeration system to avoid root damage during the treatment time.

Results: After the implementation of the sandwich system to germinate the plants, healthy (straight tap and basal roots) and homogenous plantlets were obtained (Figure 1 b and c), allowing quick selection of pairs of seedlings to be transplanted to containers with or without Al. The time to arrange and establish the whole germination system is less than 2 h, and after germination the transplanting of 120 genotypes (15 seeds x 2 treatments x 4 replicates) corresponding to circa 14,400 seeds could be completed in less than 4 h.

Physiological studies of Al resistance normally require measurements of root elongation of tap and basal roots during short periods of time (few hours) after Al treatment. Marking roots (Figure 2) 3 cm above the root tip (root zone already differentiated) just prior to the Al treatment, allows measuring the direct effect of Al on root elongation at different Al treatment times. After obtaining homogenous seedlings from the germination system, results from Figure 3 shows that no noticeable differences were observed between marked and complete root measurement methods using an Al resistant Quimbaya and Al sensitive VAX 1 genotypes. Therefore, for routine screening of Al resistance the complete root measurement could be used. However, to measure the Al effect during short or consecutive treatment times, the marking method is recommended.

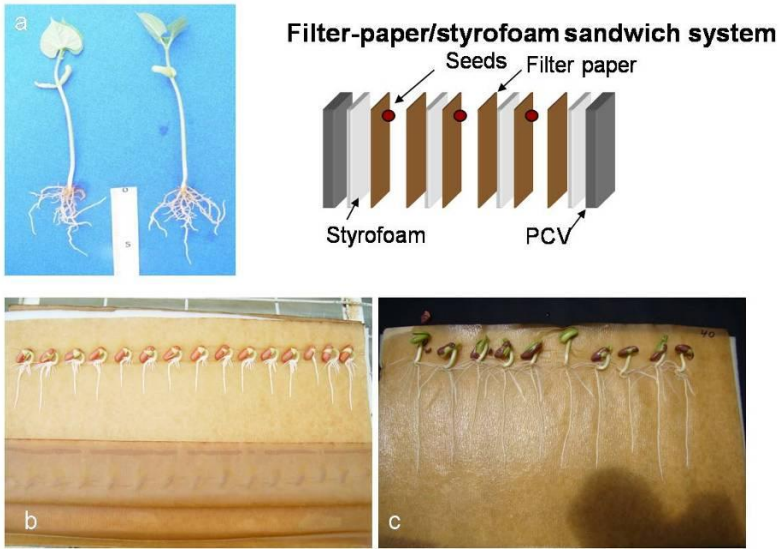


Figure 1. Comparison between seedlings germinated in sand (a) or in filter-paper/Styrofoam sandwich system after 3 (b) or 4 days (c) of germination.

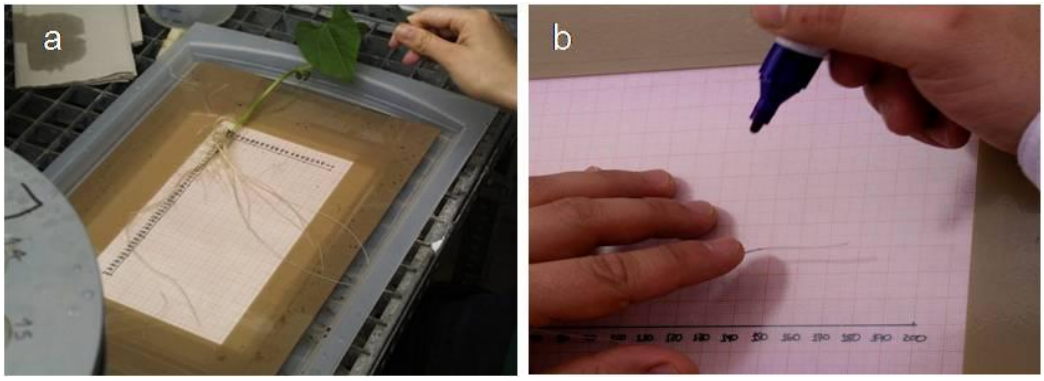


Figure 2. Root elongation measured with two methods: a) complete root and b) marking 3 cm above the root tip.

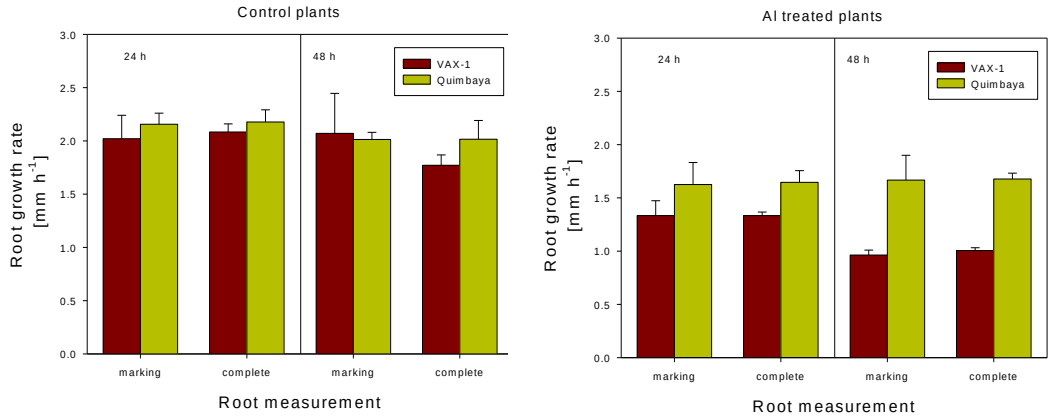


Figure 3. Influence of two different methods (marking and complete root) to measure root growth rates of two common bean genotypes (Al-resistant, Quimbaya; Al-sensitive VAX-1) after germination using filter-paper/styrofoam germination system.

Conclusions: A filter paper-styrofoam sandwich germination method was developed to improve the phenotyping capacity for Al resistance in common bean and a primary root marking method was developed to evaluate short-term effects of Al on root elongation process.

6. Qualitative indication of Al-induced citrate exudation in different *Phaseolus* species using an Agarose-Aluminon method

Contributors: A.F. Rangel, J. Ricaurte, S. Beebe, I. M. Rao and W. Horst

Rationale: Previous studies have shown that citrate exudation contributes to Al resistance in common bean. Two protocols were implemented in order to check the contribution of citrate exudation to Al resistance in different *Phaseolus* species using 1 cm excised root tips in nutrient solution (quantitative) or intact plants in agarose gels containing aluminon as color indicator (qualitative). The agarose gel technique shows that organic acid exudation occurs along the entire root system but mainly to the first 1 to 2 cm of the root tip, the most Al sensitive root zone. The amount of organic acids secreted to the agarose media of the three Phaseolus species tested decreased in the order of *P. coccineus* > *P. vulgaris* > *P. acutifolius*. The presence of Al-complexing chelators in root exudates and rhizosphere soil solution can be visualized using agarose gels, containing red-colored Al-aluminon complexes on the root surface. The presence of Al chelators with higher affinity to Al, compared to aluminon (e.g. organic acid anions, phenols) is indicated by discoloration zones.

Materials and methods: Gels of agarose (low gelling point) of 3 mm (1 % w/v) were used as carrier matrix for the Al-aluminon complex. Agarose gels were prepared in nutrient solution containing 5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3. Aluminum (250 µM) was added to the agarose containing nutrient solution after cooling down to 50^oC and adjusting the pH to 4.5. Aluminon stock solution was prepared by mixing NaOH (24g), Aluminon (175 mg) dissolved in acetic acid (120 ml) and adjusted to 500 ml with bi-distilled water and adjusting the pH to 4.2.

Different bean accessions with contrasting levels of Al-resistance were pretreated for 24 h in nutrient solution containing 20 µM of Al. This treatment time is enough to detect significant differences in Al resistance among common bean genotypes based on inhibition of root elongation. Also, it has been demonstrated that these differences corresponded with the capacity to exude organic acid anions, mainly citrate. After Al pretreatment four plants of each accession were carefully placed into flat transparent acrylic cuvettes, the root system was dispersed and a mix of 25 ml aluminon, 70 ml of agarose containing nutrient solution and Al, and 5 ml ascorbic acid (0.5%) at 40^oC, was carefully poured to cover the whole root system (3 to 4 mm thickness). Thereafter, the cuvettes were covered with plastic sheet to avoid desiccation of the agarose. Depending on the amount of Al-complexing compounds released from the root or accumulated in the rhizosphere, discoloration was visible after 6 to 8 h.

Results: In general terms, the appearance of bleached zones were observed in the complete root system but mainly in the first 1 to 2 cm root apices. Similarly, all accessions showed the capacity to exude Al-

chelating substances from the roots. However, the grade and magnitude of discolored areas were in the order of *P. acutifolius* < *P. vulgaris* < *P. coccineus*. Additionally, the extent of discolored areas were in agreement with the known Al resistance rating of all six accessions used, so less blanched areas were observed in G40159 and VAX-1, both rated as Al-sensitive. SER16, an Al-intermediate accession, showed discolored areas mainly in the first 1 to 2 cm from the root tips and in areas where more root tips appeared. G19833, Quimbaya and G35346-3Q, all classified as Al-resistant, showed a greater capacity to exude Al-chelating substances along the whole root system (Figure 1). However, the capacity to exude these substances by the coccineus accession was outstanding. The validation of this result requires quantification of the Al-chelating substances exuded from the roots. According to previous studies, this should correspond mainly to citrate exudation. Thus, this method can be used as a fast test to check the existence of citrate exudation as a mechanism of Al resistance in different bean accessions.

Conclusions: A method was adapted and validated for screening for aluminum resistance in common bean based on qualitative determination of aluminum-complexing compounds including citrate released from the roots.

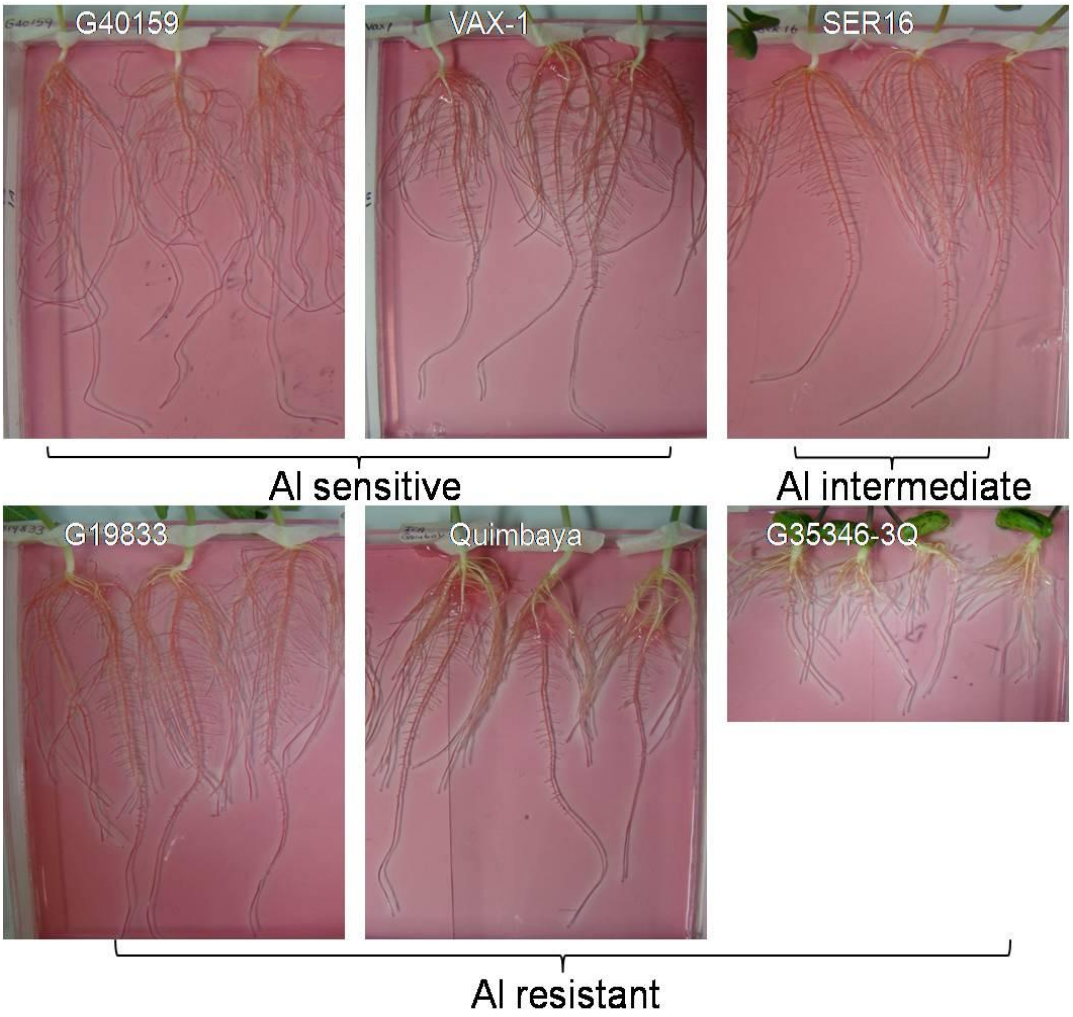


Figure 1. Qualitative determination of Al-complexing compounds (e.g. citrate) released from the roots of six bean genotypes differing in Al-resistance. G40159 (*P. acutifolius*); VAX-1, SER16, G19833 and Quimbaya (*P. vulgaris*); and G35346-3Q (*P. coccineus*).

7. Development of a greenhouse soil tube method to quantify phenotypic differences among 13 bean genotypes in root development and distribution under individual stress of high aluminum

Contributors: J. Polania, L. Butare, S. Beebe and I. M. Rao

Rationale: We developed a greenhouse soil tube method to quantify phenotypic differences in root development and distribution under simulated soil drying conditions. We adapted this method to test the effect of aluminum (Al)-toxic soil conditions on root development and distribution of 13 genotypes that were already tested for individual drought stress. Among the 13 genotypes tested included 4 accessions of *P. coccineus*.

Materials and Methods: A greenhouse study was conducted at CIAT - Palmira using an acid soil (Oxisol) from Quilichao of Colombia with a bulk density of 1.1 g cm^{-3}. Plants were grown for 34 days in small plastic cylinders (80 cm long with 7.5 cm diameter) inserted in PVC tubes from May to June 2007. The trial included 13 bean genotypes: BAT 477, BAT 881, DOR 364, G 21212, G 19833, ICA Quimbaya, VAX 1, SER 16, G 40159, G 35066 1Q, G 35884 1Q, G 35448 9P and G 35157 3Q to determine genotypic differences in root development and distribution under individual stress of high aluminum (Table 1). The trial was planted as a randomized complete block design with two levels of Al saturation: high aluminum (76% Al saturation in top soil layer and 83% in bottom soil layers) and low aluminum (28% in top soil layer and 51% in bottom soil layers) (Table 2).

Table 1. Seed color, seed size, growth habit and other characteristics of the bean genotypes grown in soil tubes in greenhouse, Palmira.

Genotype	Seed color	100 seed weight (g)	Growth habit	Nature	Genus	Species	Origin
BAT 477	Cream	21.5	3	Inbred line	*Phaseolus*	*vulgaris*	Colombia
BAT 881	Coffee	21.5	2	Inbred line	*Phaseolus*	*vulgaris*	Colombia
DOR 364	Red	23.0	2b	Inbred line	*Phaseolus*	*vulgaris*	Colombia
G 21212	Black	29.3	2	Landrace	*Phaseolus*	*vulgaris*	Colombia
G 40159	White	16.4	4	Landrace	*Phaseolus*	*acutifolius*	Mexico
G 19833	Yellow, red	43.0	3	Landrace	*Phaseolus*	*vulgaris*	Peru
G 35066	Cream	91.7	4	Landrace	*Phaseolus*	*coccineus*	Mexico
G 35884	White	133.8	4	Landrace	*Phaseolus*	*coccineus*	Macedonia
G 35448	Black	104.7	4	Landrace	*Phaseolus*	*coccineus*	Mexico
G 35157	Cream	120.3	4	Landrace	*Phaseolus*	*coccineus*	Mexico
ICA Quimbaya	Red	59.7	1	Commercial	*Phaseolus*	*vulgaris*	Colombia
SER 16	Red	25.6	2	Inbred line	*Phaseolus*	*vulgaris*	Colombia
VAX 1	Cream	29.4	3	Inbred line	*Phaseolus*	*vulgaris*	Colombia

Table 2. Aluminum saturation of an Oxisol from Quilichao in two different soil depths.

Al Level	Depth Quilichao field (cm)	Depth Soil cylinder (cm)	pH	Al Saturation (%)	P (ppm)	Organic matter (%)
High Al	0 - 10	0 – 10	4.1	76	8.8	6
High Al	10 - 20	10 – 75	4.1	83	3.3	5
Low Al	0 - 10	0 – 10	4.4	28	9.7	5.3
Low Al	10 - 20	10 – 75	4.3	51	4.3	4.5

The soil was collected from Quilichao – Colombia, in two different soils depth 0-10 cm and 10-20 cm, an acid soil with high aluminum saturation and another with low aluminum saturation. The soil with low aluminum saturation was fertilized with basal level of nutrients (kg/ha of 80 N, 50 P, 100 K, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). The cylinders were carefully packed, the bottom of the cylinder (10-75 cm) with soil from 10-20 cm of Quilichao field; and the top of cylinder (0-10 cm) with soil from 0-10 cm of Quilichao field. The plants were established with seed previously germinated on paper. Plants grown under optimal soil moisture (80% field capacity) and it was maintained by weighing each cylinder every three days and applying water to the soil at the top of the cylinder. Plants were harvested at the age of 34 days after establishment.

A number of shoot physiological characteristics were measured during the experiment including total chlorophyll content (SPAD). At the time of harvest (34 days after establishment), leaf area, shoot biomass distribution, and root traits were determined. The soil from the tube was removed and sliced into 6 layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75). Roots in each soil layer were washed free of soil and root length, mean root diameter, specific root length, and root dry weight were determined. Root length and mean root diameter were measured with an image analysis system (WinRHIZO, Regent Instruments INC). Root weight was determined after roots were dried in an oven at 60 ^{o}C for 48 h. Differences in rooting among the lines were estimated by using a model of vertical root distribution developed by Gale and Grigal (1987), which is based on the following asymptotic equation:

Y= 1-ßd

Where Y= the cumulative root biomass or root length fraction (a proportion between 0 and 1) from the soil surface to depth d (cm), and ß= the fitted "extinction coefficient". ß is the only parameter estimated in the model. It provides a simple numerical index of the root biomass or root length distribution, where high ß values (e.g., 0.98) correspond to a greater proportion of root biomass or root length at depth and low ß values (e.g., 0.91) imply a greater proportion of root biomass or root length near to soil surface. Analysis of variance was calculated by using the SAS computer program (SAS/STAT, 2001). A probability level of 0.05 was considered statistically significant.

Results and Discussion: Significant genotypic differences were observed in terms of biomass production, leaf area and root traits for both treatments: high Al and low Al. High Al markedly decreased the biomass of leaves, stem and roots of the genotypes tested when compared with low Al level. The four genotypes of *Phaseolus coccineus* G 35448 9P, G 35884 1Q, G 35157 3Q and G 35066 1Q were outstanding in their production of total biomass under high Al stress. The genotypes G 40159 (*Phaseolus acutifolius*) and SER 16 showed the lowest total biomass production under high Al stress (Figure 1). Under high Al stress the four genotypes of *P. coccineus* had the highest values of leaf area, G 35884 1Q was the best in leaf area production (Table 3). SER 16 and G 40159 also had the lowest leaf area production.

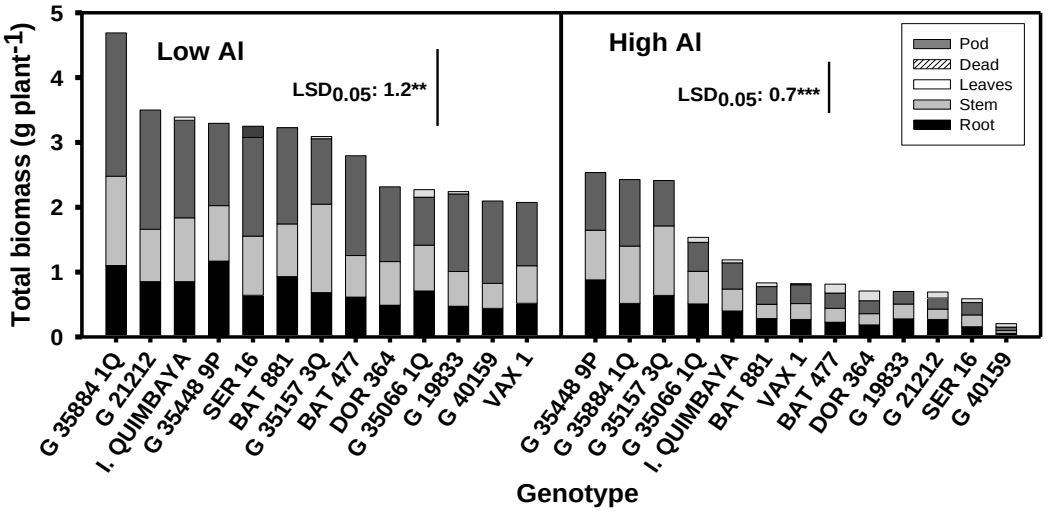


Figure 1. Dry matter distribution in 13 bean genotypes grown in soil tubes under individual stress of high aluminum.

High Al stress decreased the leaf chlorophyll content at 27 days after planting (Table 3). The genotype G 40159 had the lowest leaf chlorophyll content at 27 days after planting and G 35448 9P had the highest leaf chlorophyll content. Results on total root length showed significant genotypic variation under high Al stress (Figure 2). The four *P. coccineus* genotypes were outstanding in their total root length and also these genotypes showed lowest root length inhibition, particularly G 35448 9P, G 35257 3Q and G 35066 1Q (Figure 3). G 35448 9P was outstanding in root development under high Al stress. The genotypes SER 16 and G 40159 showed the lowest root length under high Al stress and with high values of root length inhibition (Figure 3). Significant differences were observed in terms of rooting depth at 31 days

after planting; the genotypes G 35448 9P and Ica Quimbaya were outstanding in their deep root development under high Al stress and reached the soil depth of 75 and 69 cm, respectively after 31 days of planting (Table 4). The genotypes SER 16 and G 19833 were the poor performers in terms of rooting depth by reaching soil depth of 37 and 42 cm, respectively after 31 days of planting under high Al stress.

Table 3. Influence of high and low Al saturation in soil on shoot traits of 13 genotypes of bean grown in soil tubes.

Genotype	Leaf chlorophyll content (SPAD)		Leaf area (cm^2 $plant^{-1}$)		Total biomass (g $plant^{-1}$)		Shoot to root ratio	
	High	Low	High	Low	High	Low	High	Low
BAT 477	36.0	39.9	60.1	436.4	0.81	2.79	2.61	3.57
BAT 881	36.5	36.6	83.2	451.6	0.83	3.23	1.95	2.49
DOR 364	28.8	35.6	68.4	372.2	0.71	2.32	2.89	3.66
G 19833	30.0	32.6	71.1	560.0	0.70	2.24	1.52	3.79
G 21212	29.6	37.1	63.1	615.9	0.69	3.50	1.60	3.15
G 35066 1Q	28.3	38.6	154.2	225.9	1.54	2.27	2.03	2.14
G 35157 3Q	28.4	37.0	319.5	414.7	2.41	3.09	2.94	3.48
G 35448 9P	38.1	40.1	284.5	450.7	2.53	3.29	1.87	1.87
G 35884 1Q	36.3	38.4	387.8	869.2	2.43	4.69	3.77	3.24
G 40159	17.6	41.9	18.9	388.8	0.20	2.10	2.81	3.90
I. Quimbaya	36.4	38.7	142.5	499.2	1.19	3.39	2.07	3.00
SER 16	29.8	39.2	58.7	504.3	0.59	3.25	2.79	4.06
VAX 1	32.4	37.0	101.7	347.8	0.82	2.07	2.11	3.00
Mean	**31.4**	**37.9**	**139.5**	**472.0**	**1.2**	**2.9**	**2.4**	**3.2**
$LSD_{0.05}$	**NS**	**NS**	**102*** **	**294***	**0.72*** **	**1.22** **	**0.90** **	**1.0***

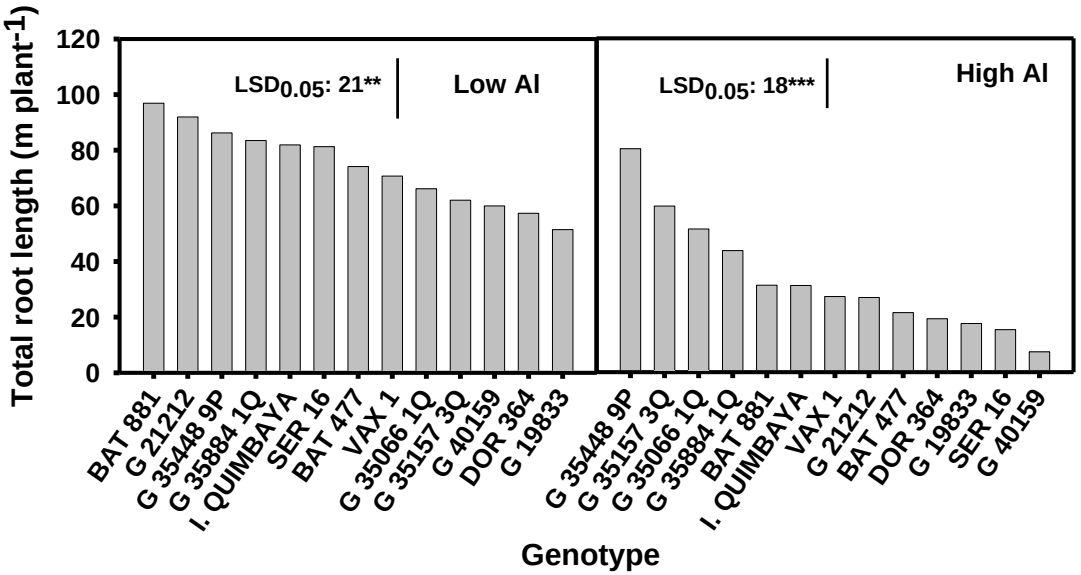


Figure 2. Influence of high and low aluminum saturation in soil on total root length in 13 genotypes of bean grown in soil tubes.

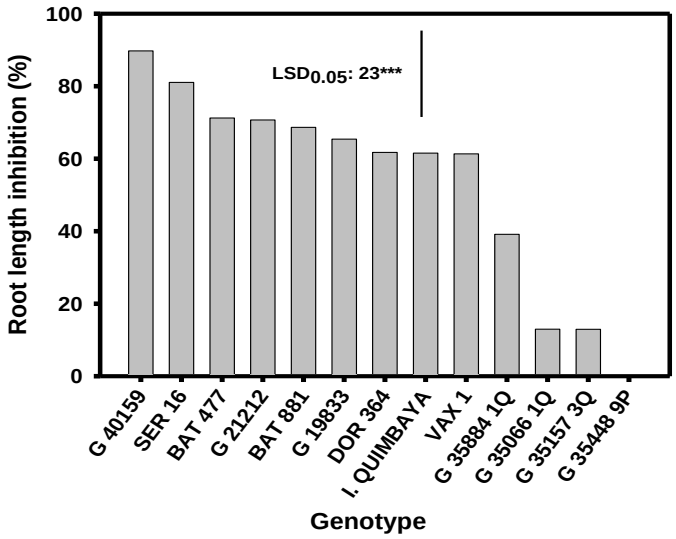


Figure 3. Root length inhibition of 13 genotypes of bean under high Al stress grown in soil tubes.

Table 4. Influence of high and low Al saturation in soil on rooting depth during active growth and development of 13 genotypes of bean grown in soil tubes. (d.a.p. = days after planting).

Genotype	Rooting depth at 10 d.a.p. (cm)		Rooting depth at 14 d.a.p. (cm)		Rooting depth at 22 d.a.p. (cm)		Rooting depth at 31 d.a.p. (cm)	
	High	Low	High	Low	High	Low	High	Low
BAT 477	14	18	23	30	39	51	51	65
BAT 881	21	21	30	33	46	43	58	50
DOR 364	16	15	25	27	37	56	44	69
G 19833	5	16	13	23	29	42	42	50
G 21212	15	17	27	33	45	64	60	75
G 35066 1Q	10	10	19	18	45	41	54	53
G 35157 3Q	8	6	16	17	47	49	61	67
G 35448 9P	16	13	30	26	61	54	75	71
G 35884 1Q	6	12	13	22	43	52	53	70
G 40159	20	24	25	33	37	53	48	71
I. Quimbaya	18	22	28	33	55	63	69	75
SER 16	12	24	18	38	29	49	37	68
VAX 1	16	16	23	24	39	55	54	75
Mean	14	16	22	27	42	52	54	66
$LSD_{0.05}$	**7.4****	**10.3***	**8.1****	**10.6***	**14.9***	**NS**	**14.9****	**NS**

Significant genotypic differences were observed in specific root length under low and high Al conditions (Figure 4). The genotype G 40159 (*P. acutifolius*) was outstanding in production of fine roots under both low and high Al conditions by showing the highest values of specific root length. Under high Al stress G 19833 and ICA Quimbaya developed thick roots, while in low Al conditions the four genotypes of *P. coccineus* had thicker roots as revealed by the lowest values of specific root length (Figure 4).

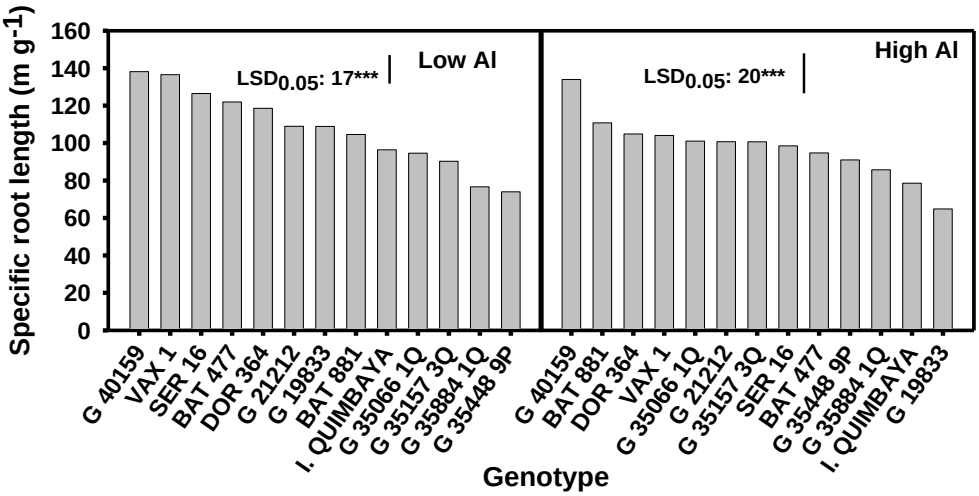


Figure 4. Influence of high and low aluminum saturation in soil on specific root length of 13 genotypes of bean grown in soil tubes.

Significant differences in root length distribution across the soil depth under individual stress of high Al were observed in all soil depths in both Al levels (Figure 5). The genotypes G 35157 3Q and G 35066 1Q had greater root length in the soil depth of 0-5 cm with 16 and 14 m plant^{-1}, respectively. G 40159 and ICA Quimbaya had the lowest root length in the soil depth of 0-5 cm soil depth with 1.6 and 3.1 m plant^{-1}, respectively. In the soil depth of 60-75 cm G 35448 9P and G 35157 3Q were outstanding in their production of root length with 9.8 and 3 m plant^{-1}, respectively while genotypes like G 40159, SER 16 and DOR 364 did not develop any roots at this soil depth under high Al stress.
Results on extinction coefficient ß in terms of length and root biomass showed significant differences under both high and low Al conditions (Table 5).

Table 5. Influence of high and low aluminum saturation in soil on root trails in 13 genotypes of bean grown in soil tubes.

Genotype	Mean root diameter (mm)		Root volume (cm^3)		Root length extinction coefficient		Root biomass extinction coefficient	
	High	Low	High	Low	High	Low	High	Low
BAT 477	0.32	0.31	2.03	6.29	0.94	0.96	0.94	0.96
BAT 881	0.31	0.32	2.50	7.75	0.95	0.96	0.95	0.96
DOR 364	0.25	0.32	1.49	4.40	0.92	0.95	0.93	0.95
G 19833	0.39	0.31	2.17	4.52	0.92	0.95	0.93	0.95
G 21212	0.33	0.33	2.36	7.98	0.95	0.96	0.95	0.96
G 35066 1Q	0.32	0.31	4.85	7.51	0.94	0.94	0.94	0.94
G 35157 3Q	0.34	0.38	6.49	7.55	0.95	0.95	0.95	0.95
G 35448 9P	0.34	0.46	7.59	13.61	0.97	0.97	0.97	0.97
G 35884 1Q	0.34	0.44	5.01	11.51	0.94	0.95	0.95	0.96
G 40159	0.23	0.30	0.49	4.02	0.93	0.97	0.93	0.97
I. Quimbaya	0.33	0.34	3.06	7.55	0.96	0.97	0.96	0.97
SER 16	0.22	0.31	1.16	5.85	0.92	0.96	0.92	0.96
VAX 1	0.31	0.31	2.13	5.02	0.92	0.96	0.93	0.96
Mean	0.31	0.34	3.18	7.20	0.94	0.96	0.94	0.96
$LSD_{0.05}$	**0.07****	**0.05*****	**2.1*****	**3.0*****	**0.02*****	**0.008*****	**0.02****	**0.008*****

Under high Al conditions the genotypes G 35448 9Q, ICA Quimbaya and G 35157 3Q presented highest values of ß with 0.97, 0.95 and 0.95, respectively for root length and the same values for root biomass,

indicating that these genotypes have greater proportion of root length and root biomass at depth. But genotypes G 40159, VAX 1 and SER 16 showed the lowest values of ß with 0.93, 0.92 and 0.92 respectively for root length and 0.93, 0.93 and 0.92 for root biomass, respectively indicating that these genotypes have greater proportion of root biomass or root length in top soil layers. Under low Al conditions the genotypes G 35448 9P, G 40159 and ICA Quimbaya had the highest values of ß with 0.97 for root length and 0.97 for root biomass. G 35066 1Q showed the lowest values of ß with 0.94 for root length and 0.94 for root biomass.

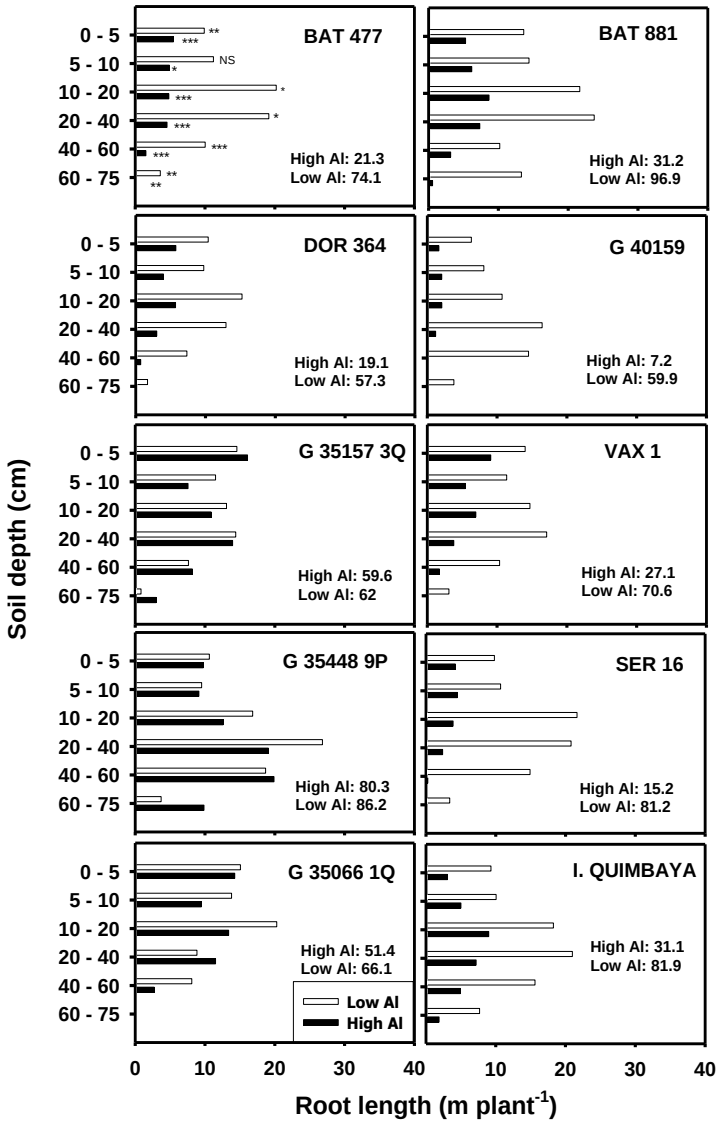


Figure 5. Influence of high and low Al saturation in soil on root length distribution across soil depth in 13 genotypes of bean grown in plastic cylinders. Palmira 2007. Total root length values across soil depth are indicated for high and low Al treatments.

Conclusions: Greenhouse evaluation to quantify phenotypic differences among 13 bean genotypes in root development and distribution under individual stress of high aluminum resulted in identification of two genotypes of *Phaseolus coccineus* G 35448 9P and G 35157 3Q that were superior in their root development. These genotypes also were superior in their production of total biomass. The genotypes G 40159 (*P. acutifolius*) and SER 16 were identified as susceptible to high Al. These genotypes had the poorest total root length and total biomass under high Al stress. In previous field and greenhouse

evaluations, SER 16 was identified as drought resistant with greater ability for photosynthate mobilization to pod and to grain together with good root development under drought stress.

8. Phenotypic differences among 16 bean genotypes in root development and distribution under drought stress

Contributors: J. Polania, L. Butare, S. Beebe and I. M. Rao

Rationale: We developed a greenhouse soil tube method to quantify phenotypic differences in root development and distribution under simulated soil drying (terminal drought) or maintainance of 40% of field capacity (intermittent drought). With this method we can simulate the drought stress effects on shoot and root development in common bean. This system is used to quantify differences among 16 genotypes in root development under drought stress. Among the 16 genotypes tested included 5 selections from G 35066 and 2 selections from G 35884 of *P. coccineus*.

Materials and Methods: A greenhouse study was conducted at CIAT - Palmira using a mix of an Andisol (from Darien of Colombia) with river sand (2:1 w/w). Plants were grown for 46 days (November 2006 to January 2007) in plastic cylinders (80 cm long with 7.5 cm diameter) that were inserted in PVC tubes. The average maximum and minimum temperature values were 35^{o}C and 21^{o}C, respectively (Figure 1) with a maximum photon flux density of 1100 µmol m^{-2} s^{-1}. Soil cylinders were carefully packed with 3320 g of soil: sand mixture, with bulk density of 1.13 g cm^{-3}. The trial included 16 bean genotypes including 8 from *P. vulgaris*: BAT 477, BAT 881, DOR 364, G 21212, G 19833, ICA Quimbaya, VAX 1, SER 16; 1 from *P. acutifolius*: G 40159 and 7 from *P. coccineus*: G 35066-1Q, G 35066-2Q, G 35066-3Q, G 35066-4Q, G 35066-5Q, G 35884-1Q and G 35884-2Q (Table 1) to determine genotypic differences in root development and distribution under drought stress. The trial was planted as a randomized complete block arrangement with three levels of water supply: 80% field capacity (well-watered), maintaining 40% field capacity (to simulate intermittent drought conditions) and withholding of watering (to simulate terminal drought stress conditions) as main plots and genotypes as sub-plots with three replications. Soil was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). Treatments of water stress were imposed after 10 days of initial growth of plants that were established with seed. The initial soil moisture for all the treatments was of 80% field capacity. Plants with well-watered treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder. Plants with intermittent drought (40% field capacity) treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder if it is necessary. Plants with terminal drought were monitored for water stress by weighing each cylinder every two days for determination of decrease in soil moisture. Plants were harvested at the age of 46 days after establishment, i.e., 36 days of withholding of water application.

A number of shoot physiological characteristics were measured during the experiment. This included total chlorophyll content (SPAD) and rooting depth. At the time of harvest (46 days after establishment; 36 days of water stress treatment), leaf area, shoot biomass distribution, and root traits were determined. The soil from the tube was removed and sliced into 6 layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75). Roots in each soil layer were washed free of soil and sand and root length, mean root diameter, specific root length, and root dry weight were determined. Root length and mean root diameter were measured with an image analysis system (WinRHIZO, Regent Instruments INC). Root weight was determined after roots were dried in an oven at 60 ^{o}C for 48 h. Differences in rooting among the lines were estimated by using a model of vertical root distribution developed by Gale and Grigal (1987), which is based on the following asymptotic equation: Y= 1-ßd Where Y= the cumulative root biomass or root length fraction (a proportion between 0 and 1) from the soil surface to depth d (cm), and ß= the fitted “extinction coefficient”. ß is the only parameter estimated in the model. It provides a simple numerical index of the root biomass or root length distribution, where high ß values (e.g., 0.98) correspond to a greater proportion of root biomass or root length at depth and low ß values (e.g., 0.91) imply a greater proportion of root biomass or root length near to soil surface. Analysis of variance was calculated by using the SAS computer program (SAS/STAT, 2001). A probability level of 0.05 was considered statistically significant.

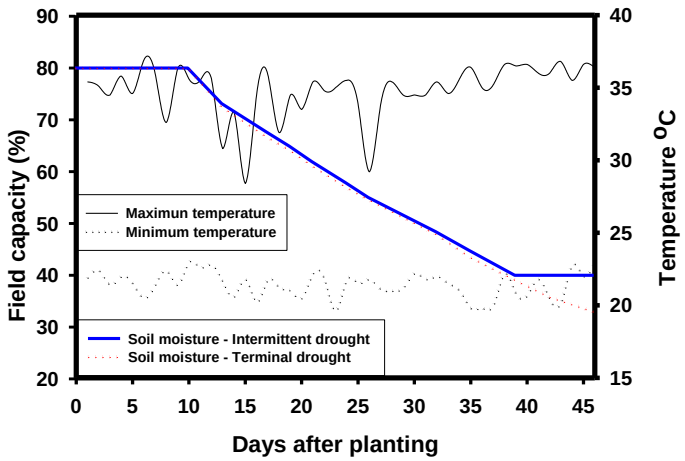


Figure 1. Soil moisture (field capacity), maximum and minimum temperatures during soil drying and root development in the soil tubes in greenhouse at Palmira.

Table 1. Seed color, seed size, growth habit and other characteristics of the bean genotypes grown in soil tubes in greenhouse, Palmira.

Genotype	Seed color	100 seed weight (g)	Growth habit	Nature	Genus	Species	Origin
BAT 477	Cream	21.5	3	Inbred line	*Phaseolus*	*vulgaris*	Colombia
BAT 881	Coffee	21.5	2	Inbred line	*Phaseolus*	*vulgaris*	Colombia
DOR 364	Red	23.0	2b	Inbred line	*Phaseolus*	*vulgaris*	Colombia
G 21212	Black	29.3	2	Landrace	*Phaseolus*	*vulgaris*	Colombia
G 40159	White	16.4	4	Landrace	*Phaseolus*	*acutifolius*	Mexico
G19833	Yellow, red	43.0	3	Landrace	*Phaseolus*	*vulgaris*	Peru
G35066	Cream	91.7	4	Landrace	*Phaseolus*	*coccineus*	Mexico
G35884	White	133.8	4	Landrace	*Phaseolus*	*coccineus*	Macedonia
ICA Quimbaya	Red	89.7	1	Commercial	*Phaseolus*	*vulgaris*	Colombia
SER 16	Red	25.6	2	Inbred line	*Phaseolus*	*vulgaris*	Colombia
VAX 1	Cream	29.4	3	Inbred line	*Phaseolus*	*vulgaris*	Colombia

Results and Discussion: The initial soil moisture level for the three treatments was of 80% field capacity. Plants under well watered (control) treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder. After 10 days plants with intermittent drought were left without irrigation until the soil moisture reached the 40% field capacity and maintained this level by applying water at the top of the cylinder, this level was reached at 38 days after planting. For plants with terminal drought stress treatment, each cylinder was weighed every two days and determined soil moisture until harvest time. The average of final soil moisture at 46 days after planting was at 32 % of field capacity (Figure 1).

Significant genotypic differences were observed in terms of biomass production in the three treatments control, intermittent and terminal water stress conditions. Water stress conditions markedly decreased the biomass of leaves, stem, pods and roots of 16 genotypes tested when compared with well-watered treatment (Figure 2). Total biomass production under intermittent drought conditions was relatively less affected for the lines SER 16, BAT 477 and G 40159 and these lines were outstanding in their production of pods under stress conditions (Table 2). Among the 16 genotypes tested under intermittent drought stress, the seven genotypes of *P. coccineus* had poor total biomass production without pod production under stress conditions. The same behavior was observed under terminal drought conditions. Three genotypes SER 16, G 40159 and BAT 477 had higher levels of total biomass and pod production.

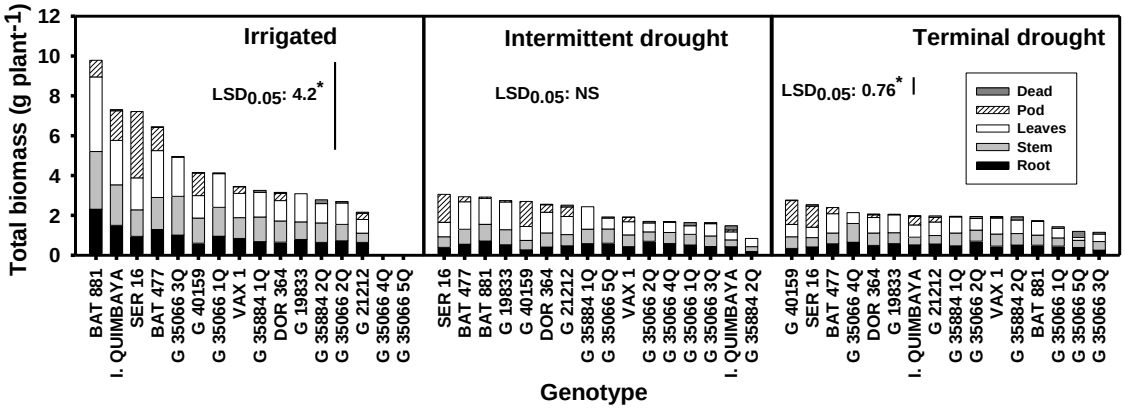


Figure 2. Dry matter distribution in 16 bean genotypes grown in soil tubes under irrigation, intermittent drought and terminal drought in greenhouse, Palmira.

Table 2. Influence of three levels of water supply on leaf area, leaf + stem biomass and pod biomass of 16 bean genotypes grown in soil tubes in greenhouse, Palmira.

Genotype	***Leaf area*** (cm^2 $plant^{-1}$)			***Leaf + stem biomass*** (g $plant^{-1}$)			**Pod biomass** (g $plant^{-1}$)		
	Irrig.	**Inter.**	**Term.**	**Irrig.**	**Inter.**	**Term.**	**Irrig.**	**Inter.**	**Term.**
BAT 477	718	412	268	3.96	2.13	1.52	1.16	0.25	0.31
BAT 881	981	379	174	6.64	2.14	1.19	0.83	0.06	0.00
DOR 364	333	308	230	2.08	1.74	1.40	0.36	0.38	0.14
G 19833	518	459	421	2.29	2.15	1.45	0	0.07	0
G 21212	231	275	192	1.15	1.47	1.10	0.31	0.48	0.23
G 40159	335	179	159	2.39	1.17	1.20	1.13	1.26	1.20
I. Qimbaya	637	134	170	4.27	0.75	1.00	1.49	0.10	0.42
SER 16	559	205	165	2.94	1.25	0.99	3.33	1.41	1.06
VAX 1	351	207	339	2.26	1.25	1.41	0.33	0.21	0.06
G 35066 1Q	619	135	167	3.14	0.97	0.89	0	0	0
G 35066 2Q	336	130	171	1.90	0.92	1.13	0	0	0
G 35066 3Q	714	206	106	3.91	1.15	0.81	0	0	0
G 35066 4Q		169	195		1.07	1.49		0	0
G 35066 5Q		149	40		1.26	0.50		0	0
G 35884 1Q	500	466	333	2.47	1.85	1.45	0	0	0
G 35884 2Q	315	141	228	1.95	0.66	1.26	0	0	0
Mean	**510**	**247**	**210**	**2.58**	**1.37**	**1.17**	**0.69**	**0.28**	**0.23**
$LSD_{0.05}$	**455***	**260***	**138*****	NS	**1.08***	**NS**	**1.28*****	**0.55*****	**0.28*****

Leaf area values under intermittent water stress simulation were greater for G 35884 1Q (*P. coccineus*) G 19833, BAT 477 and BAT 881 than the other genotypes (Table 2). ICA Quimbaya and G 35066 2Q had the lowest leaf area under intermittent drought. Under terminal drought leaf area values were greater for G 19833, VAX 1 and BAT 477. The genotype G 19833 was outstanding in leaf area production under both intermittent and terminal water stress, this genotype had a high vigor but its pod production is lower, indicating lower ability for photosynthate mobilization.

Results on total root length showed significant genotypic variation under the three water levels (Figure 3). The genotypes BAT 477 and G 19833 were outstanding in their total root length under intermittent and terminal drought. Among the 16 genotypes tested G 35066 3Q and G 35884 had poor root development under intermittent and terminal water stress conditions. Results on specific root length showed significant

genotypic differences under three treatment conditions (Figure 4). The genotypes G 40159 (*P. acutifolius*), VAX 1 and DOR 364 were outstanding in developing thin roots under intermittent and terminal water stress conditions while the seven genotypes of *P. coccineus* had thicker roots under both intermittent and terminal drought stress. This behavior can be compared with the values of the proportion of fine roots (between 0 and 0.5 mm of diameter). Results showed that the genotype G 40159 has the highest proportion of fine roots while the selections of *P. coccineus* showed lower values (Table 3).

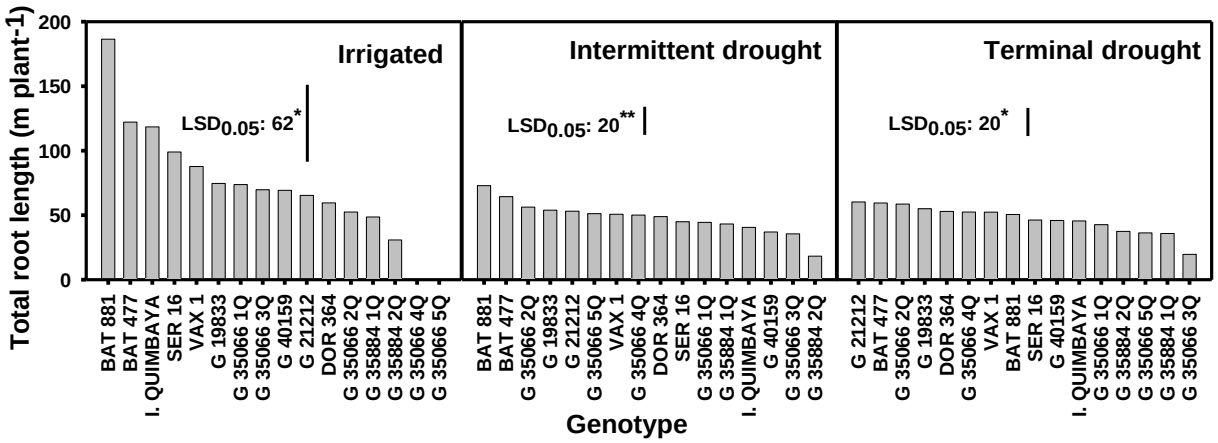


Figure 3. Total root length in 16 bean genotypes grown in soil tubes under irrigation, intermittent drought and terminal drought in greenhouse, Palmira

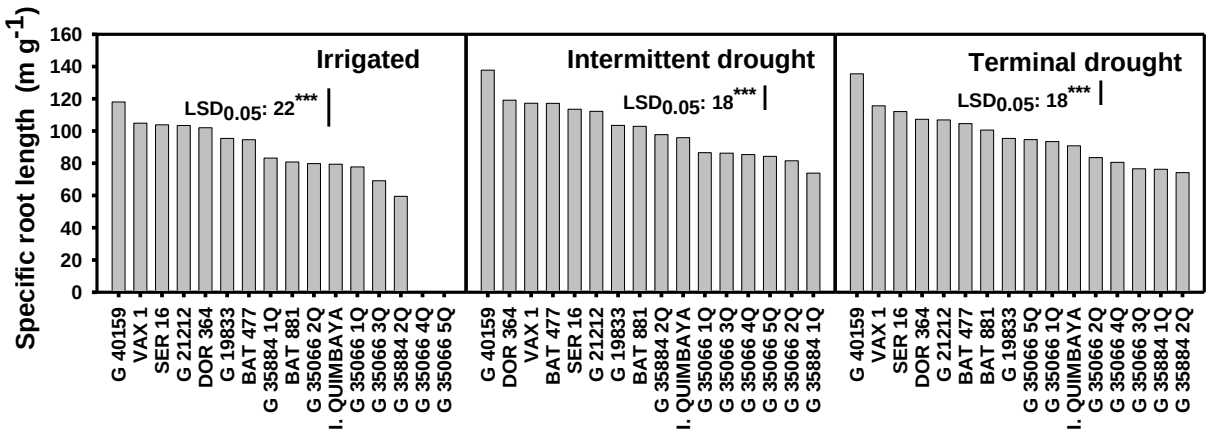


Figure 4. Specific root length of 16 bean genotypes grown in soil tubes under irrigation, intermittent drought and terminal drought stress in greenhouse, Palmira.

Table 3. Influence of three levels of water supply on root traits of 16 bean genotypes grown in soil tubes in greenhouse, Palmira.

	Proportion of fine roots (%)			Root length extinction coefficient			Root biomass extinction coefficient		
Genotype	**Irrig.**	**Inter.**	**Term**	**Irrig.**	**Inter.**	**Term.**	**Irrig.**	**Inter.**	**Term.**
BAT 477	88	89	90	0.97	0.97	0.97	0.96	0.96	0.96
BAT 881	88	90	88	0.97	0.97	0.96	0.96	0.96	0.95
DOR 364	92	92	91	0.90	0.96	0.97	0.89	0.96	0.96
G 19833	89	90	89	0.90	0.96	0.94	0.89	0.95	0.93
G 21212	90	88	87	0.92	0.96	0.97	0.92	0.96	0.96
G 40159	91	93	93	0.94	0.96	0.96	0.94	0.95	0.96
I. Quimbaya	87	84	89	0.97	0.96	0.97	0.96	0.96	0.96
SER 16	90	90	91	0.94	0.96	0.96	0.94	0.96	0.95
VAX 1	91	90	91	0.91	0.95	0.96	0.91	0.94	0.96
G 35066 1Q	87	89	87	0.95	0.94	0.93	0.94	0.93	0.91
G 35066 2Q	86	83	84	0.86	0.95	0.95	0.85	0.95	0.95
G 35066 3Q	82	86	87	0.90	0.95	0.89	0.91	0.94	0.87
G 35066 4Q		86	86		0.95	0.95		0.95	0.94
G 35066 5Q		84	86		0.95	0.91		0.95	0.90
G 35884 1Q	87	86	84	0.79	0.91	0.89	0.79	0.91	0.88
G 35884 2Q	76	89	87	0.77	0.91	0.84	0.78	0.90	0.83
Mean	**87**	**88**	**88**	**0.91**	**0.95**	**0.94**	**0.90**	**0.94**	**0.93**
$LSD_{0.05}$	**3.3*** **	**4.4** **	**3.8** **	**NS**	**0.01*** **	**0.06***	**NS**	**0.01*** **	**0.06***

Results on extinction coefficient ß in terms of length and root biomass showed significant differences under terminal drought and irrigated conditions while under intermittent drought the differences were not significant (Table 3). Under terminal drought conditions the genotypes BAT 477, DOR 364 and G 21212 presented highest values of ß with 0.97 for root length and 0.96 for root biomass, indicating that these genotypes have greater proportion of root length and root biomass at depth, while the genotypes G 35066 3Q, G 35884 1Q and G 35884 2Q showed the lower values of ß with 0.89, 0.89 and 0.84, respectively for root length and 0.87, 0.88 and 0.83 for root biomass, indicating that these genotypes have greater proportion of root biomass or root length in the top soil layers. Under irrigated conditions the genotypes BAT 477, ICA Quimbaya and BAT 881 had the highest values of ß with 0.97 for root length and 0.96 for root biomass. The lowest values of ß with 0.79 and 0.77 for root length and 0.79 and 0.78 for root biomass were observed with G 35884 1Q and G 35884 2Q, respectively.

Significant genotypic differences were observed in terms of rooting depth. Under intermittent drought the genotypes BAT 477, G 21212 and SER 16 had the highest root penetration and reached the depth of 73, 71 and 71 cm, respectively after 35 days of planting (Table 4). The development of roots under terminal drought was similar to intermittent drought. BAT 477, G 21212 and SER 16 were outstanding in their root development and reached the depth of 74, 73 and 73 cm, respectively after 35 days of planting.

Significant genotypic differences in root length distribution across the soil depth under control, intermittent and terminal water stress were found for 20-40, 40-60 and 60-75 cm soil depth (Figure 5). BAT 881 was outstanding in its root development under irrigated conditions but showed greater sensitivity to drought stress in decreasing the root development. The genotypes BAT 477 and BAT 881 had greater root length for 60-75 cm soil depth than the other genotypes under intermittent drought conditions. G 35066 1Q and G 35884 2Q had the lowest root length values in the same soil depth under intermittent drought. BAT 477 and G 21212 had greater root length at deeper soil layer of 60-75 cm than the other genotypes under terminal drought conditions. G 35884 2Q had the lowest root length in the same soil depth under terminal drought.

Table 4 Influence of three levels of water supply on rooting depth during root development of 16 bean genotypes grown in soil tubes in greenhouse, Palmira.

	Rooting depth at 17 days after planting (cm)			Rooting depth at 35 days after planting (cm)			Rooting depth at 46 days after planting (cm)		
Genotype	**Irrig.**	**Inter.**	**Term.**	**Irrig.**	**Inter.**	**Term.**	**Irrig.**	**Inter.**	**Term.**
BAT 477	25	37	34	66	73	74	73	75	75
BAT 881	31	32	32	59	68	64	75	75	61
DOR 364	29	30	30	38	64	70	50	73	75
G 19833	24	33	35	46	59	57	60	63	61
G 21212	32	33	34	41	71	73	54	75	75
G 40159	21	29	30	34	62	70	60	73	75
I. Quimbaya	22	22	33	54	61	66	75	75	63
SER 16	26	35	34	42	71	73	60	75	75
VAX 1	26	33	31	42	50	67	57	74	75
G 35066 1Q	23	26	26	43	41	46	52	65	58
G 35066 2Q	28	36	31	41	63	57	53	54	64
G 35066 3Q	30	24	25	52	52	38	55	65	46
G 35066 4Q	26	29	28	37	56	51	.	67	68
G 35066 5Q	23	28	27	.	63	45	.	55	50
G 35884 1Q	25	23	28	35	42	49	42	51	.
G 35884 2Q	27	11	21	36	37	36	56	51	65
Mean	**26**	**29**	**30**	**44**	**58**	**59**	**58**	**67**	**66**
$LSD_{0.05}$	**NS**	**8.7*****	**NS**	**20.9***	**NS**	**16.8*****	**NS**	**NS**	**10.9*****

Correlation coefficients between pod biomass and other shoot attributes under terminal drought conditions indicated that greater pod biomass was positively related to specific root length, proportion of fine roots and rooting depth at 35 days after planting (Table 5). Root biomass extinction coefficient was also positively associated with pod biomass. Under intermittent drought conditions significant positive relationship was observed between pod biomass and specific root length, root biomass and root length extinction coefficient, proportion of fine roots and rooting depth at 35 days after planting. This observation indicates that the superior performers developed greater amount of fine and deep roots.

Table 5. Correlation coefficients (r) between pod biomass (g $plant^{-1}$) and other plant attributes of 16 genotypes of bean grown under three levels of water supply in greenhouse, Palmira.

Plant traits	**Irrigated**	**Intermittent drought**	**Terminal drought**
Leaf area (cm^2 $plant^{-1}$)	0.26	0.14	-0.17
Leaf biomass (g $plant^{-1}$)	0.29	0.19	-0.03
Stem biomass (g $plant^{-1}$)	0.33*	0.08	-0.003
Root biomass (g $plant^{-1}$)	0.31	-0.30	-0.26
Total biomass (g $plant^{-1}$)	0.66***	0.57***	0.64***
Root length (m $plant^{-1}$)	0.49**	-0.034	0.025
Specific root length (m g^{-1})	0.25	0.63***	0.62***
Mean root diameter (mm)	0.09	-0.23	-0.02
Proportion of fine roots (m $plant^{-1}$)	0.26	0.48**	0.50***
Rooting depth at 35 days after planting (cm)	0.26	0.37*	0.44**
Root length extinction coefficient	0.33*	0.35*	0.29
Root biomass extinction coefficient	0.35*	0.33*	0.31*

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

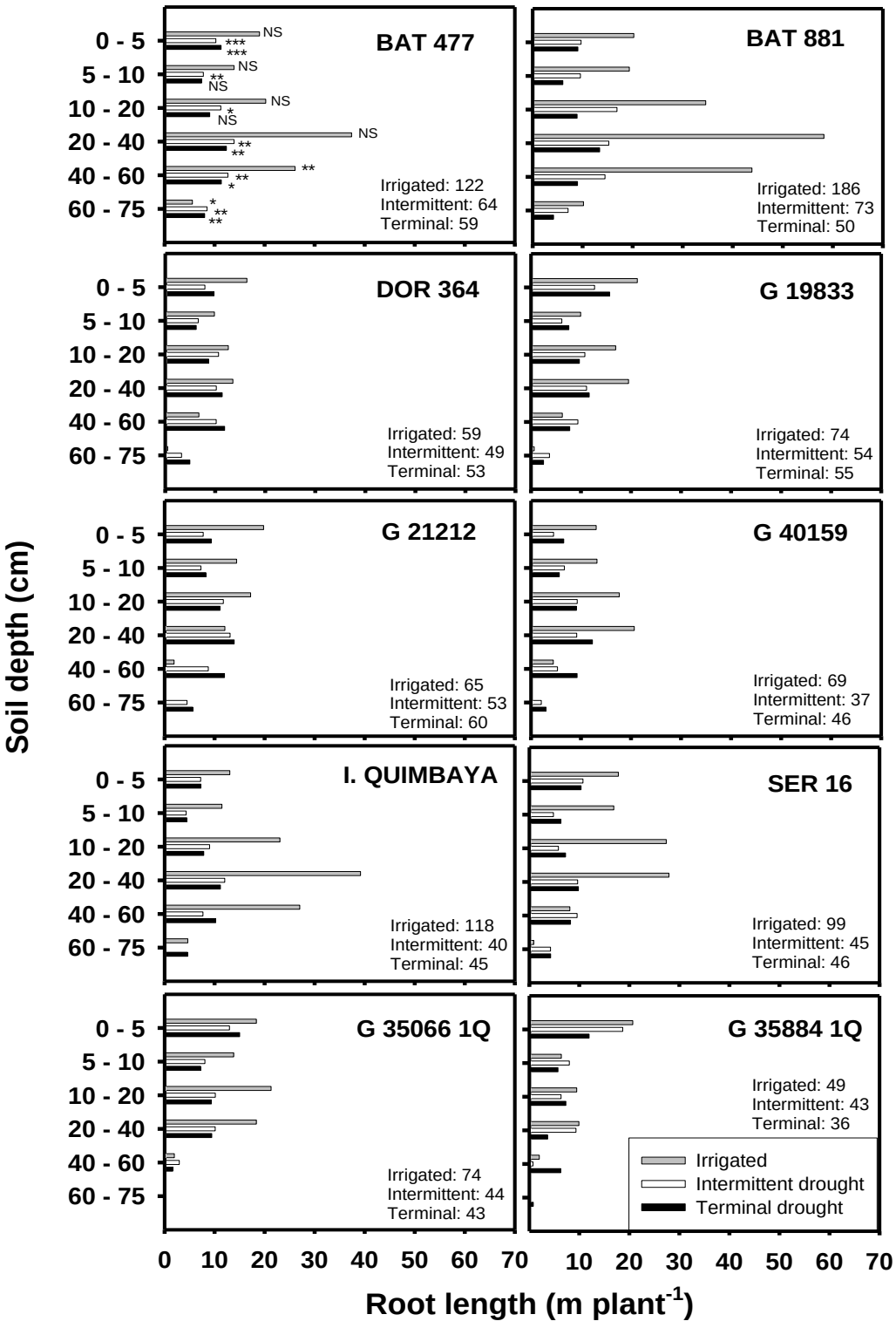


Figure 5. Influence of three levels of water supply on root length distribution across soil depth in 16 bean genotypes grown in plastic cylinders, Palmira. Total root length values across soil depth are indicated for control (irrigated) and stress treatments.

Conclusions: Greenhouse evaluation of 16 bean genotypes using soil tube method for root phenotyping resulted in identification of three genotypes BAT 477, G 21212 and G 19833 that were superior in their root development under intermittent and terminal drought stress conditions. The lines BAT 477 and G 21212 also had greater values of rooting depth under drought stress conditions. This evaluation also resulted in identification of the genotype G 40159 (*P. acutifolius*) that was superior in its development of fine roots under drought stress. Greenhouse evaluation of these lines for phenotypic differences in root development and distribution indicated that the recently developed SER 16 line could combine fine root development across soil layers with photosynthate mobilization ability that was observed under field conditions.

9. Influence of individual and combined stress of high aluminum and drought in an acid soil on root development and distribution of different species of *Phaseolus*

Contributors: J. Polania, L. Butare, S. Beebe and I. M. Rao

Rationale: Previous research indicated that aluminum (Al) toxicity reduces root elongation and the simulated terminal drought stress induces deep rooting ability in common bean genotypes. Higher level of Al resistance was observed in some accessions of runner bean (*P. coccineus*) while greater level of drought resistance and deeper and finer rooting ability were observed with tepary bean (*P. acutifolius*) accession. Although the phenotypic differences in root development among different *Phaseolus* species for these individual stress factors was evaluated before, the phenotypic response to combined stress factors was not quantified. Therefore the objective of this work was to evaluate the influence of combined stress factors of high Al toxicity and drought in an acid soil on root development and distribution of eleven genotypes of different *Phaseolus* species grown in soil tubes under greenhouse conditions.

Materials and methods: A greenhouse study was conducted at CIAT - Palmira using an acid soil (from Quilichao of Colombia). Plants were grown for 35 days in soil tubes made with plastic cylinders (80 cm long with 7.5 cm diameter) that were inserted in PVC tubes. The trial included 11 bean genotypes including 2 genotypes of *Phaseolus coccineus* (G 35066 5Q, G 35884 1Q), 8 genotypes of *Phaseolus vulgaris* (BAT 477, BAT 881, DOR 364, G 19833, G 21212, ICA Quimbaya, SER 16 and VAX 1), and 1 genotype of *Phaseolus acutifolius* (G 40159). The trial was planted as a randomized complete block arrangement with two levels of Al saturation: low Al (with a pH of 4.6 and Al saturation of 12.5%) and high Al (with a pH 4.1 and Al saturation of 77%) and two levels of water supply: 80% field capacity (well-watered) and withholding of watering (to simulate terminal drought stress conditions) as main plots and genotypes as sub-plots with three replications. Soil with low Al saturation was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). Treatments of water stress were imposed after 8 days of initial growth of plants that were established with seed. The initial soil moisture for all the treatments was of 80% field capacity. Plants with well-watered treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder. Plants with terminal drought were monitored for water stress by weighing each cylinder every two days for determination of decrease in soil moisture. Plants were harvested at 35 days after establishment, i.e., 27 days of withholding of water application.

A number of shoot traits were measured during the experiment, including total chlorophyll content (SPAD) and rooting depth. At harvest time (35 days after planting; 27 days with water stress treatment), leaf area, shoot biomass distribution, and root traits were determined. The soil from the tube was removed and sliced into 6 layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75 cm). Roots in each soil layer were washed free of soil and sand and root length, mean root diameter, specific root length, and root dry weight were determined. Root length and mean root diameter were measured with an image analysis system (WinRHIZO, Regent Instruments INC). Root weight was determined after roots were dried in an oven at 60 ^{o}C for 48 h.

Results and discussion: During the plant growth and development the maximum and minimum air temperatures were 32 and 19 ^{o}C (Figure 1). The final soil moisture was at 28% of field capacity. No significant genotypic differences were observed in shoot biomass production among treatments except in low aluminum + terminal drought. The genotypes SER 16 and G 35884 1Q were outstanding in their shoot biomass production under single terminal drought stress (Figure 2). Under high aluminum stress two genotypes of *Phaseolus coccineus* (G 35066 5Q, G 35884 1Q) produced greater amounts of shoot biomass. The same two *P. coccineus* accessions were also outstanding in shoot biomass production under combined high aluminum and drought stress. The genotypes with poor shoot biomass production under drought stress were G 35066 5Q and G 19833. Under high aluminum stress BAT 881 and G 19833

showed lower values of shoot biomass while under combined high aluminum and drought stress VAX 1 and BAT 881 produced lower shoot biomass.

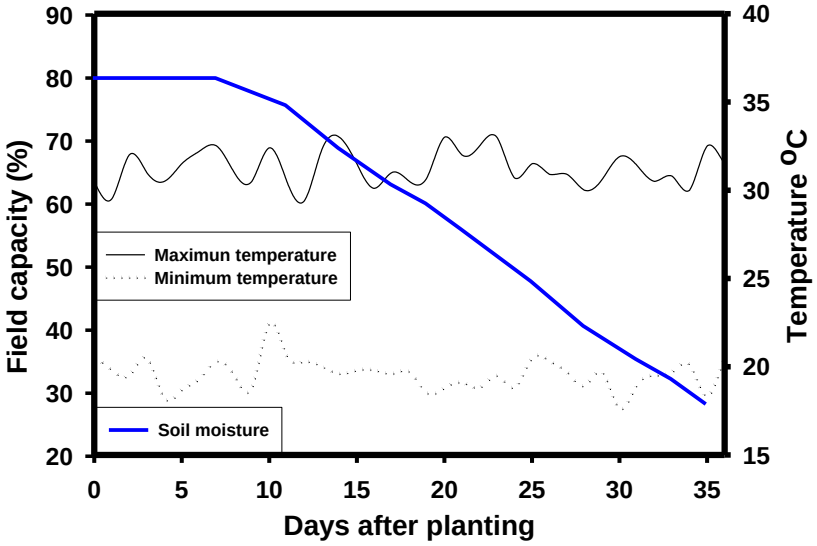


Figure 1. Soil moisture (field capacity), maximum and minimum temperature during soil drying and root development in soil tubes under greenhouse conditions at CIAT, Palmira.

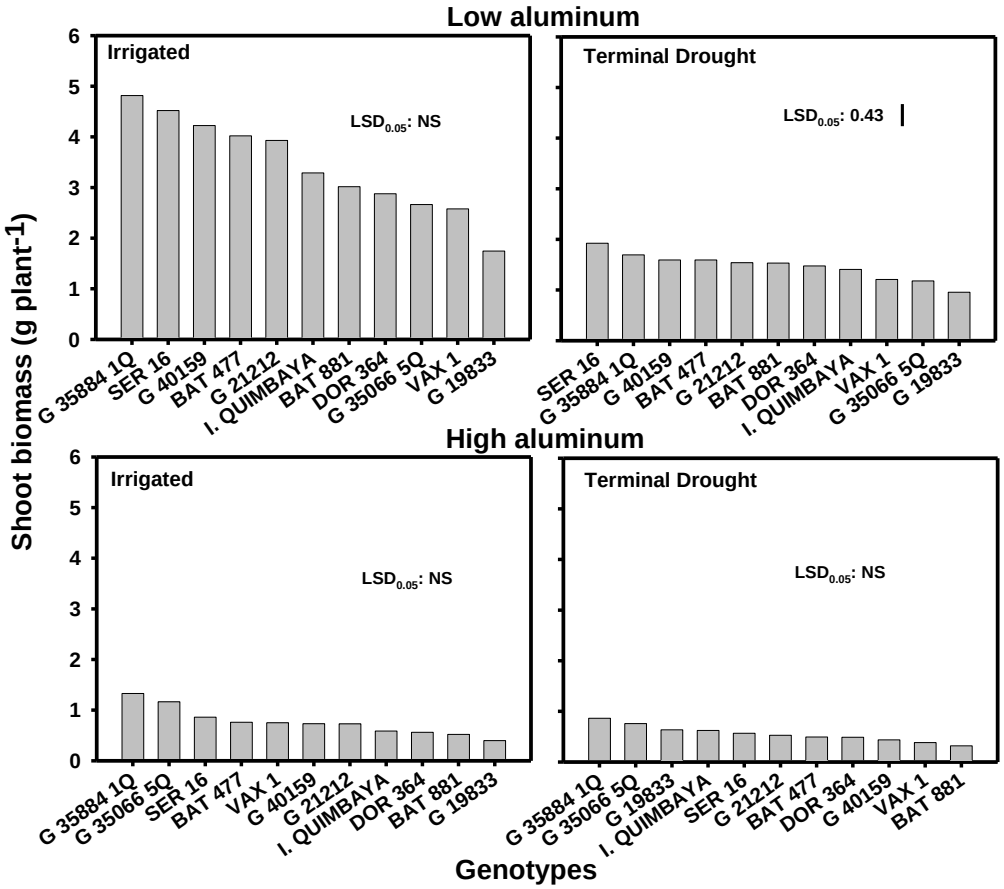


Figure 2. Influences of individual and combined stress of high aluminum and drought on shoot biomass production of 11 bean genotypes grown under greenhouse conditions at CIAT-Palmira.

In general, greater root development in deeper soil layers was observed with drought stress while Al toxicity reduced the root development in deeper soil layers (Table 1). No significant genotype differences were observed in deep rooting at 24 days after planting within treatments except for low aluminum + drought. However, two genotypes (BAT 477 and G 35066 5Q) were found to be outstanding in their deep rooting ability under combined high aluminum and drought stress (Table 1). Under high aluminum alone stress, two accessions of *Phaseolus coccineus* (G 35066 5Q, G 35884 1Q) showed greater ability for deep rooting among the genotypes tested. Three lines, ICA Quimbaya, SER 16 and BAT 477, showed deep rooting ability under terminal drought stress alone.

Table 1. Influence of individual and combined stress factors of high aluminum and drought on deep rooting ability at 24 days after planting of 11 bean genotypes grown in soil tubes under greenhouse conditions at CIAT-Palmira.

Genotype	Deep rooting at 24 days after planting (cm plant^{-1})			
	High aluminum		Low Aluminum	
	Irrigated	Drought	Irrigated	Drought
BAT 477	35	55	66	73
BAT 881	37	45	59	69
DOR 364	43	42	55	70
G 19833	36	32	54	34
G 21212	45	43	65	72
G 35066 5Q	**54**	**54**	**66**	**66**
G 35884 1Q	**45**	**35**	**60**	**67**
G 40159	41	29	67	72
I. Quimbaya	39	52	68	75
SER 16	33	51	72	75
VAX 1	34	45	62	67
Mean	**40**	**44**	**63**	**67**
$LSD_{0.05}$	**NS**	**NS**	**NS**	**14**

No significant genotypic differences in total root length were observed under individual and combined stress of high aluminum and drought. Two genotypes of *Phaseolus coccineus* (G 35066 5Q, G 35884 1Q) were superior in total root length production under high aluminum stress alone and the combined stress of high aluminum and drought while G 19833 showed lower values of total root length under individual stress of drought or high aluminum stress conditions (Figure 3). Under drought stress alone, four genotypes of *P. vulgaris* (SER 16, ICA Quimbaya, G 21212 and BAT 477) were found to be superior in total root length production. Although G 40159 was superior in total root length under terminal drought alone with low Al, it performed poorly under high Al and the combined stress of high Al and drought.

Genotypic differences were observed in mean root diameter under individual and combined stress of high aluminum and drought (Figure 4). Two accessions of *Phaseolus coccineus* (G 35066 5Q and G 35884 1Q) were found to be outstanding in thick root development under individual and combined stress of high aluminum and drought. The genotype G 19833 developed thin root system but under the combined stress of high aluminum and drought developed thicker root system (Figure 4). Under terminal drought stress alone with low Al, the genotypes SER 16 was outstanding in fine root production than the other genotypes. Two lines, BAT 881 and VAX 1, developed finer root system (lower values of mean root diameter) under combined stress of high aluminum and drought.

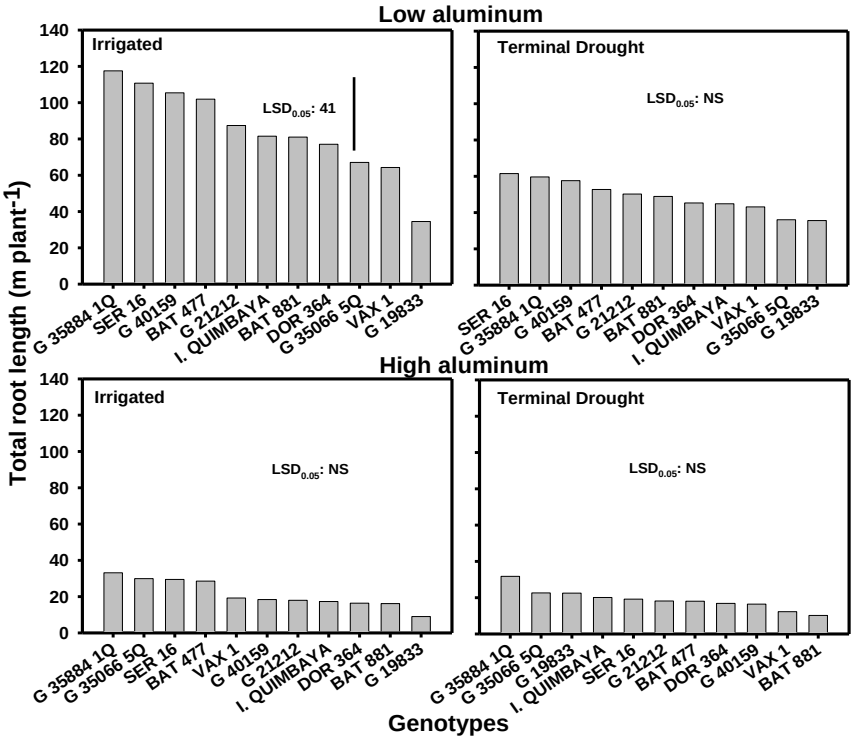


Figure 3. Influence of individual and combined stress of high aluminum and drought on total root length of 11 bean genotypes grown under greenhouse conditions at CIAT-Palmira.

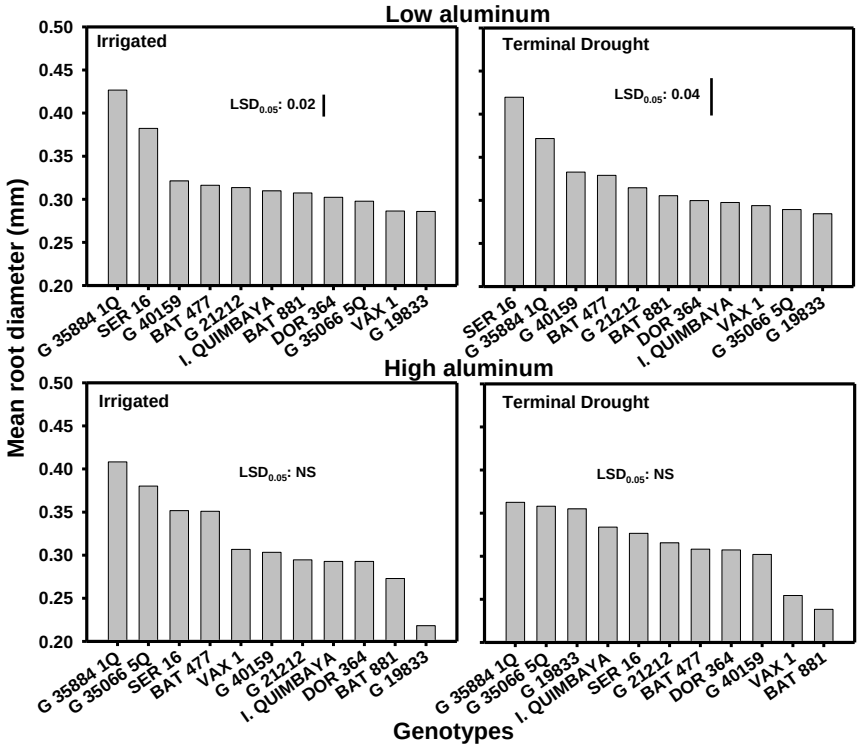


Figure 4. Influence of individual and combined stress of high aluminum and drought on mean root diameter of 11 bean genotypes grown under greenhouse conditions at CIAT-Palmira.

High aluminum stress alone and the combined stress of high aluminum and drought caused a marked inhibition in root elongation that resulted in lower values of root length at soil depth of 60-75 cm in the soil tube for all the genotypes (Figure 5). Although genotypic differences under these two stress conditions were not significant, *P. coccineus* genotype G 35884 1Q performed slightly better than other genotypes. It appears from these results that the effect of combined high aluminum and drought stress on root development was mainly due to Al inhibited root elongation process. Further work is needed in which the level of Al saturation in soil can be lowered to 60% and then evaluate the combined stress effect on genotypic differences in deep root development.

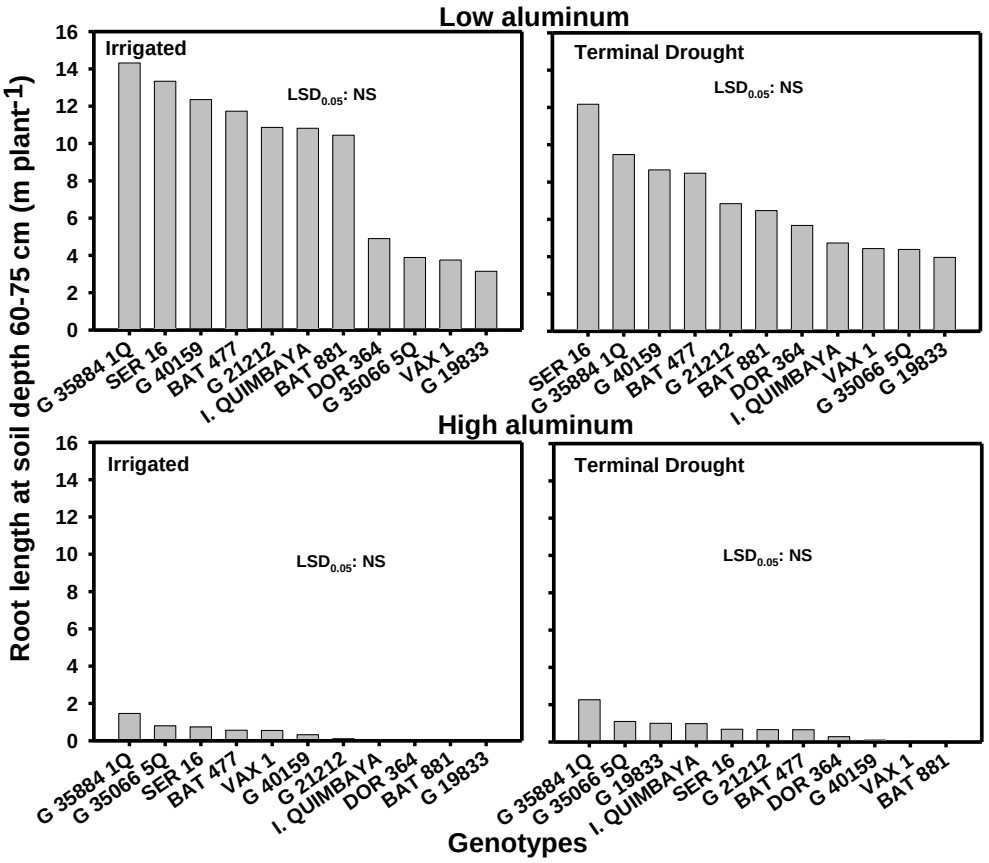


Figure 5. Influence of individual and combined stress of high aluminum and drought on root length at soil depth of 60-75 cm in 11 bean genotypes grown in plastic cylinders under greenhouse conditions, CIAT, Palmira 2008.

Conclusions: Results from this greenhouse study to determine the influence of combined stress factors of high aluminum and drought in an acid soil on root development and distribution of eleven genotypes of different *Phaseolus* species grown in soil tubes under greenhouse conditions indicated that one genotype of *Phaseolus coccineus* (G 35884 1Q) was superior in root development under high aluminum stress and combined stress of high aluminum and drought.

10. Phenotypic differences in acid soil adaptation and low phosphorus tolerance of elite lines of common bean

Contributors: J. Ricaurte, M.A.Grajales, R. García, S. Beebe and I. M. Rao

Rationale: Common bean production in the tropics is affected by three major abiotic stress factors. These include drought, low phosphorus (P) availability in soil, and aluminum (Al) toxicity in acid soils. Field evaluation of a set of elite lines including checks at CIAT-Palmira over two seasons under intermittent drought stress resulted in identification of six small seeded common bean lines (NCB 226, SEN 56, SER 113, SER 125, SXB 415 and SER 16) that were outstanding in their adaptation to drought stress conditions. The superior performance of these lines under drought stress was associated with

higher values of harvest index, pod harvest index, leaf area index and canopy biomass. The same set of elite lines including checks was evaluated under aluminum-toxic acid soil conditions and low P soil conditions to identify genotypes that are adapted to multiple abiotic stress conditions and to identify plant attributes that contribute to multiple abiotic stress adaptation. Development of multiple abiotic stress tolerant genotypes is an integral part of developing eco-efficient common bean genotypes for bean based agricultural systems.

Materials and Methods: A set of 36 bean genotypes including germplasm accessions and breeding lines, using cowpea as a check, was planted at two field sites in Colombia (March to May 2008 at Quilichao and November 2008 to February 2009 at Darién). Soil chemical characteristics of Quilichao (990 masl; 26 ^{o}C average temperature; Oxisol – Plinthic Kandiudox) field site at two soil depths (0-10 cm and 10-20 cm, respectively) showed low pH (4.61 and 4.84), moderately higher levels of exchangeable Al (45% and 58% of Al saturation), low availability of Ca (2.1 and 1.66 $cmol_c\ kg^{-1}$) and Mg (0.70 and 0.51 $cmol_c\ kg^{-1}$), adequate levels of K (0.28 and 0.18 $cmol_c\ kg^{-1}$) and P (12 and 7 $\mu g\ g^{-1}$, Bray II). The field trial at Quilichao was fertilized (kg ha^{-1}) with 2.4 of P, 100 of MgSO4 and 50 of Agrimins (N 8%, P 2.2%, Ca 13%, Mg 3.6%, S 1.6%, B 1%, Zn 2.5%). Soil chemical characteristics of Darién (1550 masl; Inceptisol) field site at two soil depths (0-10 cm and 10-20 cm, respectively) showed moderate acidity (pH 5.5 and 5.6) and very low P availability (Bray II: 2.9 and 1.8 $\mu g\ g^{-1}$). The trial at Darién was fertilized with 15 kg ha^{-1} of P as triple super phosphate.

Field trials at Quilichao and Darién included a set of 36 bean genotypes from the breeding program, grouped into 4 historical checks (BAT 477, San Cristóbal 83 [G 17722], SEA5, SEA 15), 3 small seeded red parents (Tío Canela 75, EAP 9653-16B-1, EAP 9503-32B), 11 small seeded red lines (SER 109, SER 119, SER 128, SER 16, SER 125, SER 118, SER 78, RCB 273, SER 90, SER 113, SER 48), 1 black seeded parent (DOR 390), 4 black seeded lines (NCB 280, NCB 226, SEN 36, SEN 56), 3 carioca parents (Carioca, Perola, A 686, A 774), 5 carioca lines (SXB 418, SXB 405, SXB 409, SXB 412, SXB 415), 2 wild *P. vulgaris* accessions (G 24390, G 19902), 1 *P. acutifolius* accession (G 40001) and 1 *Vigna unguiculata* genotype (Cowpea Mouride). A number of plant attributes were measured at mid-pod filling (leaf area index, total chlorophyll content-SPAD, photosynthetic efficiency, canopy temperature, canopy temperature depression, stomatal conductance, shoot biomass, shoot N and P uptake as well as shoot TNC content. Measurements made at harvest time included grain yield, pod number per area, seed number per area, 100 seed weight, seed N content, seed P content and seed TNC content. Days to flowering and days to maturity were also recorded.

Results and Discussion: Results on weather conditions at Quilichao (acid soil) and Darién (low P soil) are shown in Figure 1. During the crop-growing season, Quilichao had a total rainfall of 574 mm while Darén had 298 mm. The mean maximum temperature was 28.3 °C at Quilichao and 23.1 °C at Darién. The difference between mean maximum and mean minimum temperature was 10.1 °C at Quilichao and 6.4 °C at Darién. Two short dry spells were observed at Darién between 15 to 33 days and 36 to 50 days after planting.

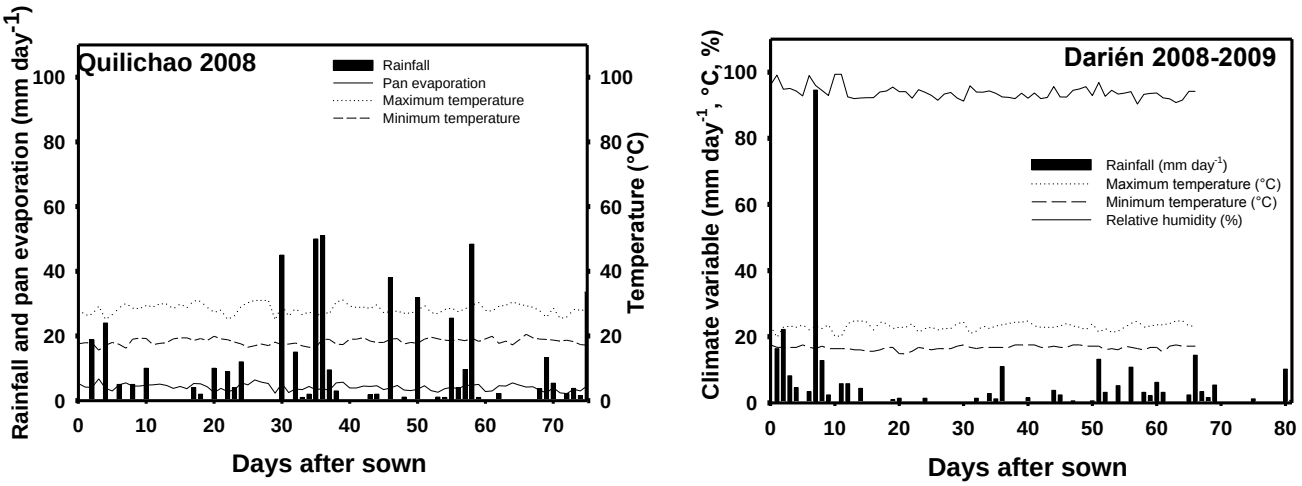


Figure 1. Rainfall distribution, pan evaporation, maximum and minimum temperatures and relative humidity during crop growing period at two field sites of Quilichao (acid soil) and Darién (low P soil).

Significant genotypic variation in grain yield was observed under both acid soil and low P soil conditions (Figure 2). Two Mulatinho lines (SXB 412, SXB 405), two carioca lines (SXB 409, SXB 415) and two small seeded red lines (SER 113, SER 118) were found to be superior in grain yield under both acid soil and low P soil conditions. The mean value of grain yield under low P soil conditions was greater than that of acid soil conditions. The two wild *P. vulgaris* accessions (G 24390, G 19902) were yielded very low under both soil conditions. One accession of *P. acutifolius* also yielded low compared with most of the elite lines. Among the elite lines, SXB 412 (Mulatinho) was particularly outstanding in grain yield under low P soil conditions while SER 118 (small red) was outstanding under acid soil conditions. Cowpea genotype outperformed the bean genotypes under acid soil conditions but its performance under low P soil conditions was below the mean value for all genotypes. Elite line SER (small red) 16 which is very well adapted to drought stress conditions showed below the mean value for both soil conditions. Elite line SXB 409 (Carioca) appears to be better adapted to both soil conditions based on grain yield.

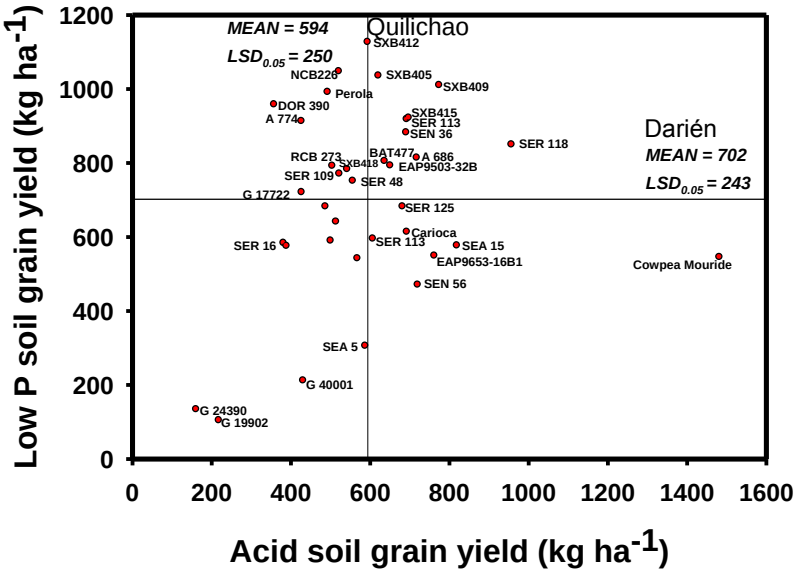


Figure 2. Identification of common bean genotypes adapted to acid soil and low P soil conditions. Genotypes with greater grain yield in both conditions are identified in the upper right hand quadrant.

Relationship between acid soil grain yield and acid soil shoot biomass indicated that the great vigor of cowpea contributed to its superior grain yield (Figure 3A). Among the elite lines SER 118 had greater grain yield but only moderate level of shoot biomass while SEN 56 had greater shoot biomass than SER 118 but its grain yield was lower than SER 118. The superior performance of SER 118 under acid soil conditions was associated with higher value of pod harvest index indicating the importance of photosynthate mobilization to grain (Figure 3B). Other elite lines that showed higher values of pod harvest index lacked the vigor needed to achieve higher grain yield under acid soil conditions. Among the elite lines, SEN 56 (small black) was outstanding in its production of pod number per area and seed number per area indicating its improved sink strength under acid soil conditions (Figure 4).

Under low P soil conditions, SXB 412 (Mulatinho) was outstanding in its shoot biomass production and grain yield while NCB 226 produced higher grain yield with below average shoot biomass production indicating its greater ability to mobilize photosynthates to grain (Figure 5A). The superior ability of NCB 226 (small black) in mobilizing photosynthates to grain was reflected through its higher value of pod harvest index (Figure 5B). The two wild *P. vulgaris* accessions (G 24390, G 19902) showed very low values of pod harvest index.

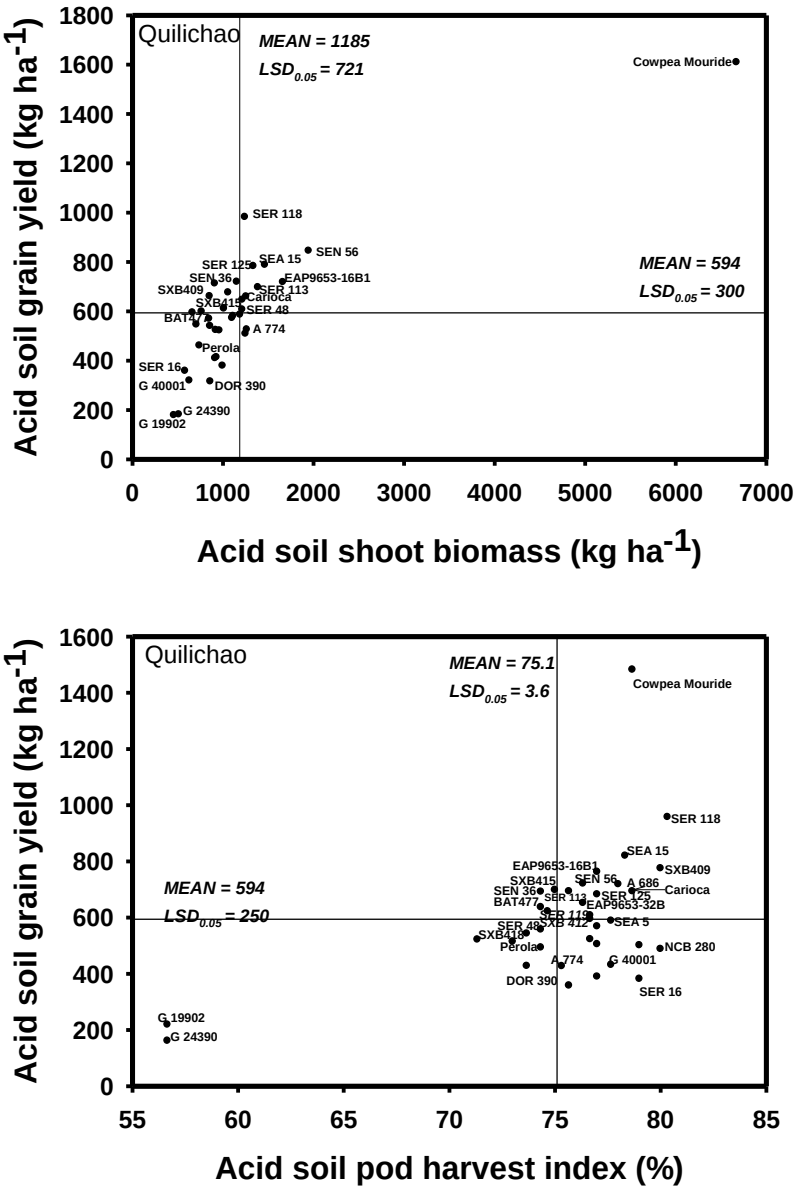


Figure 3. Identification of common bean genotypes that are adapted to acid soil and have higher shoot biomass production, pod harvest index and resulted in higher grain yield when were evaluated in an Oxisol of Quilichao. Better genotypes are identified in the upper right hand quadrant.

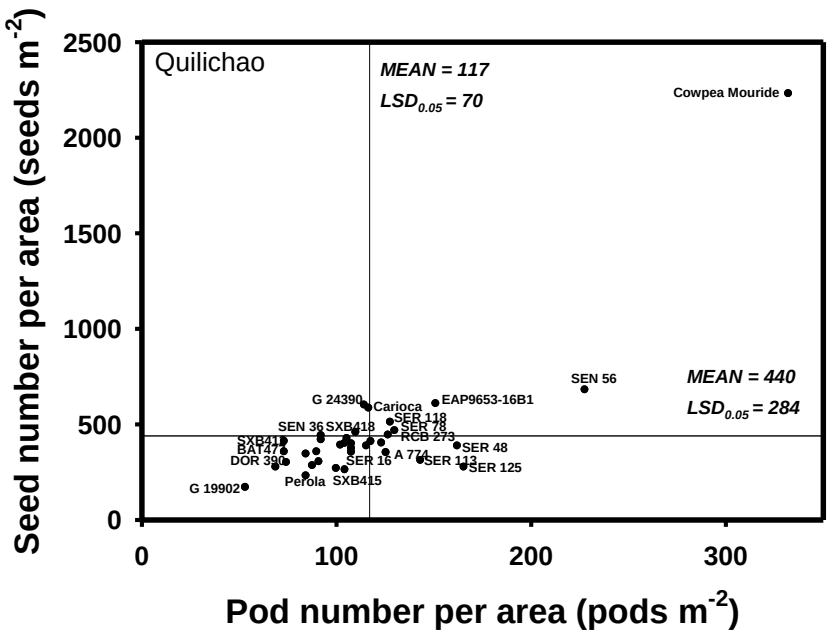


Figure 4. Identification of common bean genotypes with higher values of pod number per area and seed number per area when grown in an acid soil at Quilichao. Genotypes with greater values of pod and seed number per area are identified in the upper right hand quadrant.

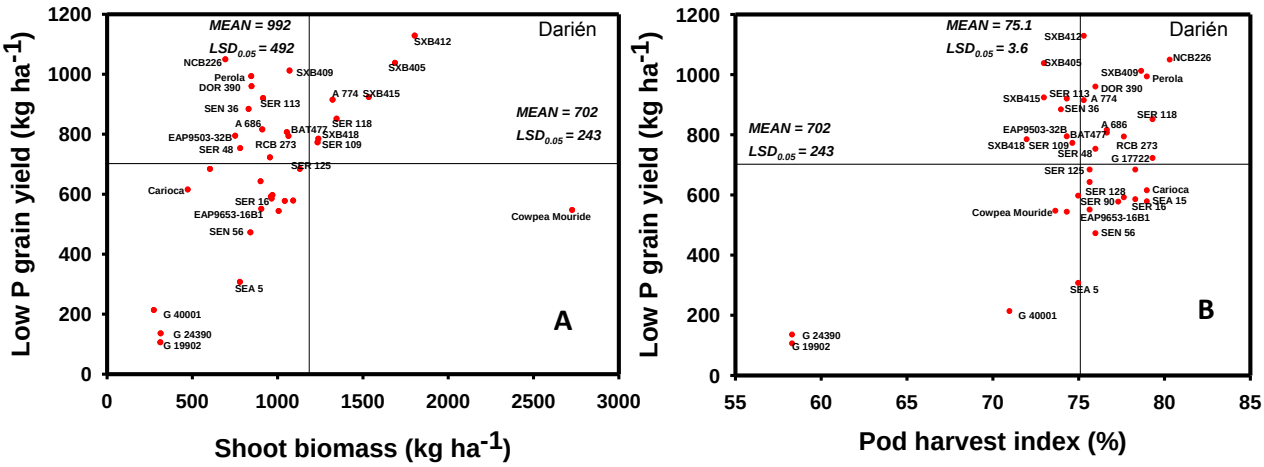


Figure 5. Identification of common bean genotypes that are adapted to low P available in soil and have higher shoot biomass production, pod harvest index and resulted in higher grain yield when were evaluated in an Inceptisol in Darién. Better genotypes are identified in the upper right hand quadrant.

Correlation coefficients between grain yield and other plant attributes are shown in Table 1. Under acid soil conditions, grain yield showed significant positive association with leaf area index, shoot biomass, pod number per area, seed number per area, pod harvest index, shoot N uptake and shoot P uptake (Table 1). Under low P soil conditions, grain yield showed significant positive association with pod harvest index, 100 seed weight, shoot biomass, shoot N uptake, shoot P uptake and seed TNC content. Seed P content showed significant negative association with grain yield (Table 1).

Table 1. Correlation coefficients (r) between grain yield (kg ha^{-1}) and other plant attributes of 36 bean genotypes that were evaluated in an acid soil (Quilichao) and low P soil (Darién).

Plant traits	Acid soil	Low P soil
Leaf area index (m^2 m^{-2})	0.76***	0.10
Total chlorophyll content (SPAD)	0.05	-0.20*
Photosynthetic efficiency (Fv'/Fm')	0.25**	0.07
Stomatal conductance (mmol m^{-2} s^{-1})	0.32***	
Shoot biomass (kg ha^{-1})	0.74***	0.42***
Pod harvest index (%)	0.39***	0.49***
Pod number per area (pods m^{-2})	0.54***	0.07
Seed number per area (seeds m^{-2})	0.66***	0.16
100 seed weight (g 100 $Seeds^{-1}$)	0.11	0.49***
Days to flowering (Days)	0.27**	0.08
Days to maturity (Days)	0.20*	0.18
Shoot N uptake (kg ha^{-1})	0.73***	0.41***
Seed N content (%)	0.07	-0.28**
Shoot P uptake (kg ha^{-1})	0.75***	0.44***
Seed P content (%)	-0.45***	-0.43***
Seed TNC content (mg g^{-1})		0.33***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

These relationships indicate that genotypes that acquire higher amounts of N and P and use these nutrients to produce moderate to high vigor and mobilize greater proportion of photosynthates to grain are better adapted to either acid soil or low P soil conditions. Shoot P uptake showed greater positive association with grain yield in acid soil conditions while greater seed filling (as reflected by 100 seed weight) and seed TNC content showed greater positive association with grain yield under low P soil conditions. Significant negative association of seed P content with grain yield under both acid soil and low P soil indicate that P use efficiency played a greater role under both stress conditions.

Conclusions: Field evaluation of thirty six bean genotypes including elite lines for their adaptation to acid soil and low phosphorus soil resulted in identification of two Mulatinho lines (SXB 412, SXB 405), two carioca lines (SXB 409, SXB 415), and two small seeded red lines (SER 113, SER 118) that were found to be superior in grain yield. Their superior performance was associated with higher values of pod harvest index and lower seed P content under both acid soil and low P soil conditions.

Brachiaria

1. Phenotypic differences in aluminum resistance of selected Brachiaria genotypes

Contributors: J. Ricaurte, R. García, J. W. Miles and I. M. Rao

Rationale: For the last seven years, we have implemented a screening procedure using hydroponics to identify aluminum (Al)-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. In 2005, we evaluated the BR04NO series of 139 apomictic/sexual hybrids of *Brachiaria* and identified 9 hybrids (BR04NO/1018, BR04NO/1552, BR04NO/1900, BR04NO/2110, BR04NO/2128, BR04NO/2166, BR04NO/2179, BR04NO/2201 and BR04NO/2681) that were superior to the *B. decumbens* parent in terms of Al resistance. In 2006, we evaluated 103 clones of the BR05NO series, 60 clones of the RZ05NO series, and 88 clones of the SX05NO series together with 3 parents and 8 checks for their level of Al resistance. Among the 103 hybrids (apomictic/sexual) of the BR05 population evaluated, nine hybrids (BR05NO/0406, BR05NO /0563, BR05NO /0334, BR05NO /0830, BR05NO /1173, BR05NO /0671, BR05NO /0120, BR05NO /0048, and BR05NO /0537) were superior to the *B. decumbens* parent in terms of root length with Al in solution. Among the 88 hybrids (sexual) of SX05 population evaluated, none was superior to the *B. decumbens* parent in terms of total root length with Al in solution but 2 sexual hybrids (SX05NO/1953 and SX05NO/1968) were superior in their ability to produce fine roots in the presence of Al in solution compared to the rest of the hybrids tested. In 2007, we evaluated 192 clones of the cross between *B. ruziziensis* x *B. decumbens* for phenotypic differences in Al resistance. We found transgressive segregation for Al resistance as was observed before with another population of the same cross. Several clones were found to be superior in their level of Al resistance than the Al resistant parent, *B. decumbens*. These data are being used to identify QTLs related to Al resistance in *Brachiaria*. This year we evaluated three groups of preselected genotypes from previous populations including 31 (SX03, SX05, BR02, BR04 and BR05 and checks), 79 (MX02, BR02, BR04, BR05, BR06 and checks); and 43 (31 SX05 and 12 checks) with and without Al in solution for their level of Al resistance.

Materials and methods: Three groups of Brachiaria hybrids generated from the breeding program along with checks were evaluated under hydroponic conditions for their level of Al resistance. A first group of three incomplete sets (separate experiments) of 31 *Brachiaria* genotypes (3 SX03, 8 SX05, 4 BR02, 3 BR04, 5 BR05 and 8 checks), a second group of three incomplete sets (separate experiments) of 79 genotypes (10 MX02, 5 BR02, 8 BR04, 20 BR05, 28 BR06 and 8 checks), and a third group of four incomplete sets (separate experiments) of 43 genotypes (31 clones of the SX05 population and 12 checks) were evaluated with 0 and 200 µM of Al in solution for their level of Al resistance under greenhouse conditions at CIAT-Palmira. The sets were incomplete because some of the hybrids did not root well in each experiment. Mean values from all the experiments are reported for each group of genotypes. Stem cuttings of all genotypes were rooted in a low ionic strength nutrient solution in the glasshouse for nine days. Equal numbers of stem cuttings with about 5 cm long roots were transferred into a solution containing 200 µM $CaCl_2$ pH 4.2 (reference treatment) and a solution containing 200 µM $CaCl_2$ and 200 µM $AlCl_3$ pH 4.2 (Al treatment). The solutions were changed every second day to minimize pH drifts. At harvest, on day 21, after transfer, root systems were harvested. Roots were scanned on a flatbed scanner with transparency unit (EPSON 4800). Image analysis software (WinRHIZO v 2003b) was used to determine root length and average root diameter.

Results and discussion

As reported in previous years, Al resistant clones combine greater values of total root length per plant with lower values of mean root diameter relative to the mean values of the population when exposed to 21 days with toxic level of Al in solution. We found significant phenotypic variation in total root length and mean root diameter under both without and with Al treatment (Figures 1 and 2).

Total root length of the 31 *Brachiaria* genotypes was markedly decreased with Al (Table 1, Figure 1). The mean root length was 415 cm $plant^{-1}$ under without Al treatment and this value decreased to 234 cm $plant^{-1}$ with Al treatment showing a reduction of 44%. The mean root diameter increased from 0.355 mm to 0.446 mm (31%) with exposure to Al (Table 1, Figure 2). The decrease in root length and increase in root diameter with Al exposure is due to Al toxicity effect on root elongation process.

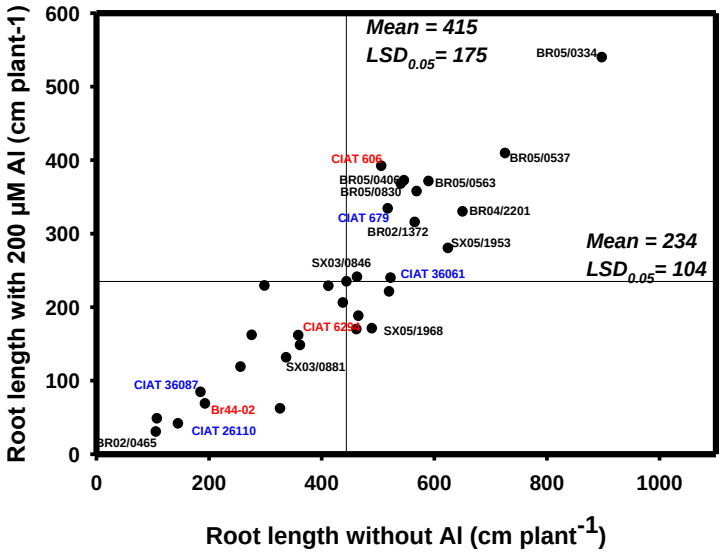


Figure 1. Relationship between total root length with Al and total root length without Al in solution of 31 *Brachiaria* gentoypes. Gentoypes that developed greater root length under both conditions were identified in the upper, right hand quadrant.

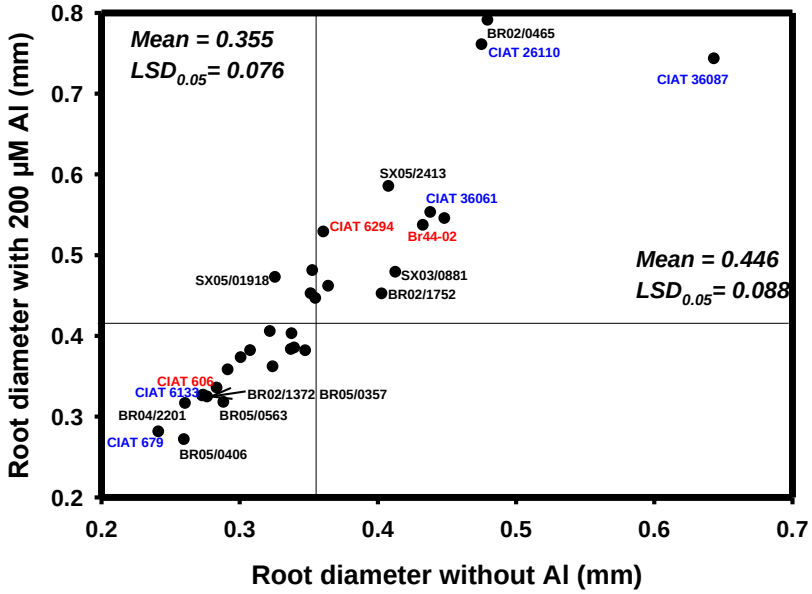


Figure 2. Relationship between mean root diameter with Al and mean root diameter without Al in solution of 31 *Brachiaria* gentoypes. Gentoypes that developed finer roots under both conditions were identified in the lower, left hand quadrant.

Two apomictic hybrids (BR05NO/0334 and BR05NO/0537) were superior to apomictic parent *B. decumbens* CIAT 606 in terms of root length with and without Al in solution (Table 1, Figures 2 and 3). Two sexual hybrids (SX05NO/1953 and SX03NO/0846) were superior to the sexual parent *B. ruziziensis* 44-02 in terms of root length with and without Al in solution (Table 1, Figures 2 and 4).

Table 1. Root length and mean root diameter of 31 *Brachiaria* genotypes evaluated with (200 µM Al) and without Al (0 µM Al) in solution

Genotypes	Root length (cm)		Average diameter (mm)	
	0 Al	200 µMAl	0 Al	200 µMAl
BR05NO/0334	898	539	0.340	0.385
BR05NO/0537	726	409	0.274	0.326
Bdec CIAT606	507	391	0.284	0.335
BR05NO/0406	547	372	0.260	0.271
BR05NO/0563	591	371	0.289	0.317
BR05NO/0830	542	367	0.292	0.358
BR04NO/2201	569	357	0.261	0.316
Bhum CIAT679	518	333	0.242	0.281
Bdic CIAT6133	651	329	0.273	0.325
BR02NO/1372	566	315	0.277	0.324
SX05NO/1953	625	280	0.308	0.382
SX03NO/0846	464	240	0.337	0.383
CIAT36061	523	239	0.438	0.553
BR04NO/2681	299	229	0.324	0.361
SX05NO/2167	413	228	0.348	0.381
BR04NO/2110	521	221	0.301	0.373
BR02NO/1485	439	206	0.352	0.452
BR02NO/1752	466	188	0.403	0.452
SX05NO/1968	490	170	0.322	0.405
Bbriz CIAT6294	463	169	0.361	0.528
SX05NO/2313	277	161	0.338	0.402
SX05NO/2413	359	161	0.408	0.585
SX05NO/1918	363	148	0.326	0.472
SX03NO/0881	338	131	0.413	0.479
SX05NO/2332	257	118	0.364	0.461
CIAT36087	186	84	0.644	0.743
Bruz 44-02	194	68	0.433	0.537
SX05NO/2547	327	61	0.353	0.481
SX03NO/2367	108	48	0.449	0.545
Bbriz CIAT26110	146	41	0.475	0.760
BR02NO/0465	106	30	0.480	0.791
Mean	**445**	**234**	**0.355**	**0.446**
$LSD_{0.05}$	***175***	***104***	***0.076***	***0.088***

Total root length of the 79 *Brachiaria* genotypes was markedly decreased with Al (Table 2, Figure 5). The mean root length was 451 cm plant^{-1} under without Al treatment and this value decreased to 295 cm plant^{-1} with Al treatment showing a reduction of 35%. The mean root diameter increased from 0.355 mm to 0.446 mm (13%) with exposure to Al (Table 2, Figure 6). Five apomictic hybrids (BR06NO/1278, BR06NO/0531, BR06NO/0012, BR06NO/1175 and BR05NO/0334) were superior to apomictic parent *B. decumbens* CIAT 606 in terms of root length with and without Al in solution (Table 2, Figure 5). Two of them (BR06NO/0012 and BR06NO/1175) generated finer root system than *B. decumbens* CIAT 606 with high Al in solution (Table 2, Figure 6).

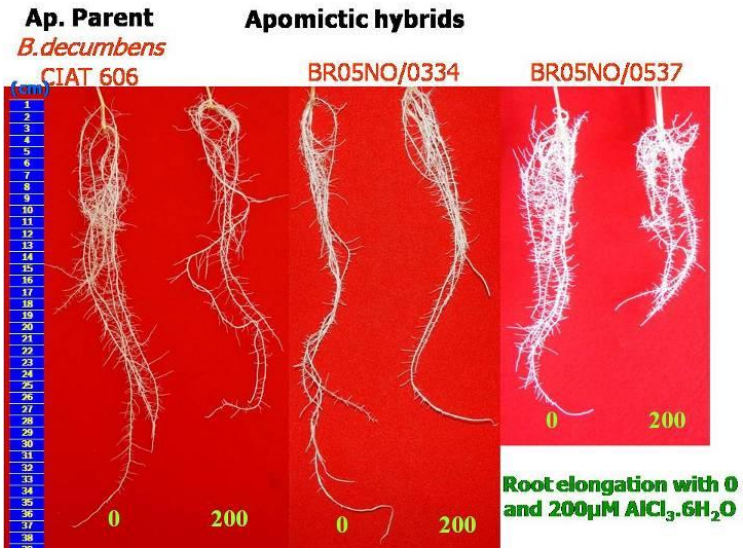


Figure 3. Two outstanding apomictic *Brachiaria* hybrids in root length production with 0 and 200 µM Al in solution, compared with their apomictic parent.

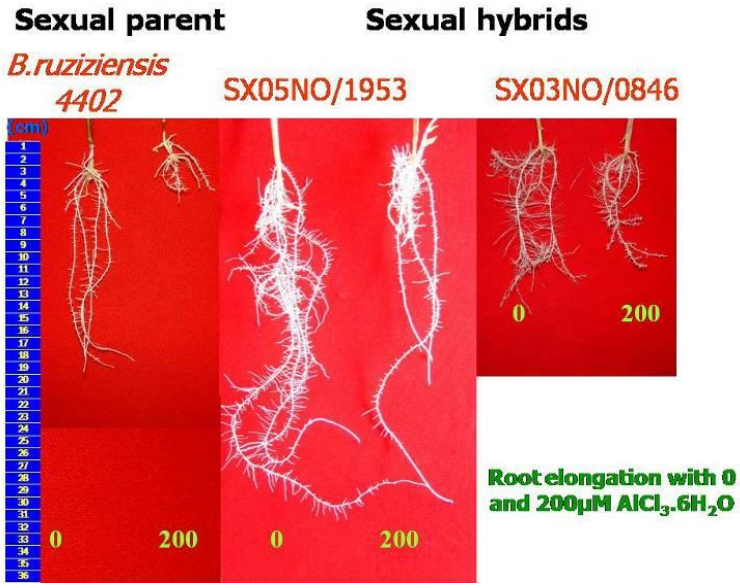


Figure 4. Two outstanding sexual *Brachiaria* gentoypes in root length production with 0 and 200 µM Al in solution, compared with their sexual parent.

Table 2. Root length and mean root diameter of 79 *Brachiaria* genotypes evaluated with (200 µM Al) and without Al (0 µMAl).

Genotypes	Root length (cm plant^{-1})		Average diameter (mm)		Genotypes	Root length (cm plant^{-1})		Average diameter (mm)	
	0 Al	200µMAl	0 Al	200µMAl		0 Al	200µMAl	0 Al	200µMAl
BR06NO/1278	518	611	0.3106	0.3268	BR06NO/1415	527	275	0.2880	0.3302
BR06NO/0531	727	600	0.3260	0.3611	MX02NO/03641	455	270	0.3990	0.4041
BR06NO/0012	778	585	0.2888	0.3051	BR06NO/1454	479	269	0.3147	0.3643
BR06NO/1175	757	566	0.2811	0.3074	BR05NO/01609	447	268	0.2998	0.3335
BR05NO/00334	636	553	0.3544	0.3673	BR05NO/01435	408	266	0.2930	0.3396
Bdec CIAT606	606	480	0.2805	0.3182	BR06NO/2058	318	262	0.3823	0.4171
BR06NO/0423	706	469	0.2966	0.3194	BR06NO/1388	414	261	0.2866	0.3155
BR06NO/0387	379	455	0.3080	0.3251	BR05NO/00743	360	258	0.2950	0.3206
BR06NO/2020	659	448	0.3291	0.3321	BR05NO/01520	389	256	0.3568	0.4188
BR06NO/0204	613	446	0.3189	0.3292	BR06NO/1433	344	235	0.2757	0.3056
BR05NO/00537	784	436	0.2878	0.3370	MX02NO/03626	347	233	0.3802	0.4339
BR04NO/02069	520	429	0.2856	0.3135	MX02NO/03426	471	231	0.4238	0.4714
BR06NO/1348	466	423	0.3240	0.3264	BR05NO/01449	344	213	0.3338	0.4061
BR04NO/01018	503	417	0.3042	0.3444	MX02NO/02552	272	211	0.4152	0.4483
BR05NO/00637	497	408	0.2817	0.3371	BR05NO/00931	452	206	0.2958	0.3377
BR05NO/00563	674	406	0.2668	0.2894	BR06NO/1567	334	196	0.3121	0.3703
BR06NO/1922	495	400	0.2885	0.3252	BR04NO/02405	372	191	0.4012	0.4379
BR06NO/0850*	530	394	0.2575	0.3033	BR02NO/1794	436	188	0.3999	0.4458
BR06NO/1696	662	381	0.2878	0.2935	BR04NO/03025	234	183	0.2817	0.3297
CIAT36061	634	368	0.4352	0.4849	BR05NO/01426	328	178	0.3491	0.3770
BR05NO/00744	460	350	0.2753	0.3096	BR05NO/01738	238	175	0.3234	0.3812
BR06NO/0584	466	347	0.2949	0.3456	Bbriz CIAT6294	343	168	0.3062	0.4076
BR06NO/1254	338	346	0.3588	0.3670	BR05NO/01469	439	164	0.2990	0.4514
BR06NO/1000	678	345	0.2765	0.3106	BR04NO/01061	331	150	0.3211	0.4299
BR05NO/01702	418	331	0.3209	0.3588	BR04NO/02515	336	148	0.3179	0.4143
BR02NO/1372	456	324	0.2775	0.3041	MX02NO/01614	242	143	0.3508	0.3859
BR05NO/00760	417	320	0.2872	0.3284	Bdic CIAT6133	218	139	0.2729	0.2734
BR06NO/2204	537	316	0.3052	0.3476	MX02NO/01769	349	123	0.3129	0.3741
BR05NO/01467	520	315	0.3101	0.3569	Bruz 44-02	225	113	0.4068	0.4053
BR06NO/1832	582	314	0.2918	0.3200	BR05NO/01706	244	107	0.2696	0.3067
BR05NO/01586	467	311	0.3089	0.3703	MX02NO/02090	177	105	0.3405	0.3943
BR06NO/1932	385	310	0.3281	0.3466	BR04NO/03207	149	104	0.2978	0.3785
BR06NO/0206	493	298	0.3223	0.3509	CIAT36087	180	103	0.5408	0.6425
BR06NO/1132	630	293	0.2877	0.3285	MX02NO/03731	202	98	0.5290	0.5623
BR05NO/00549	684	287	0.2939	0.3730	BR04NO/02774	255	91	0.3681	0.4749
BR02NO/1752	595	287	0.4121	0.4282	MX02NO/02531	79	91	0.4865	0.4480
BR06NO/1366	517	285	0.3343	0.3641	BR02NO/1718	229	82	0.4593	0.5508
MX02NO/02775	325	277	0.3565	0.3894	Bbriz CIAT 26110	75	49	0.5142	0.6797
BR06NO/0405	364	276	0.3248	0.3313	BR02NO/0465	87	33	0.4215	0.6752
Bhum CIAT679	626	276	0.2267	0.2816					
					Mean	**451**	**295**	**0.3317**	**0.3740**
					LSD0.05	***138***	***101***	***0.0410***	***0.0513***

* This clone was not a hybrid. It was later identified as *B. decumbens* CIAT 606.

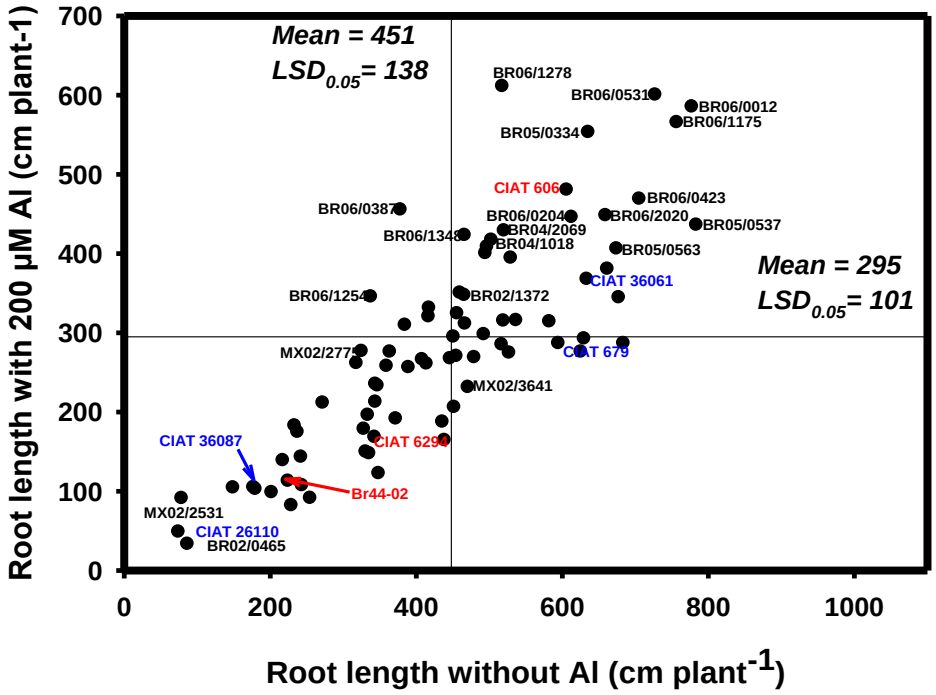


Figure 5. Relationship between total root length with Al and total root length without Al in solution of 79 *Brachiaria* gentoypes. Gentoypes that developed greater root length under both conditions were identified in the upper, right hand quadrant.

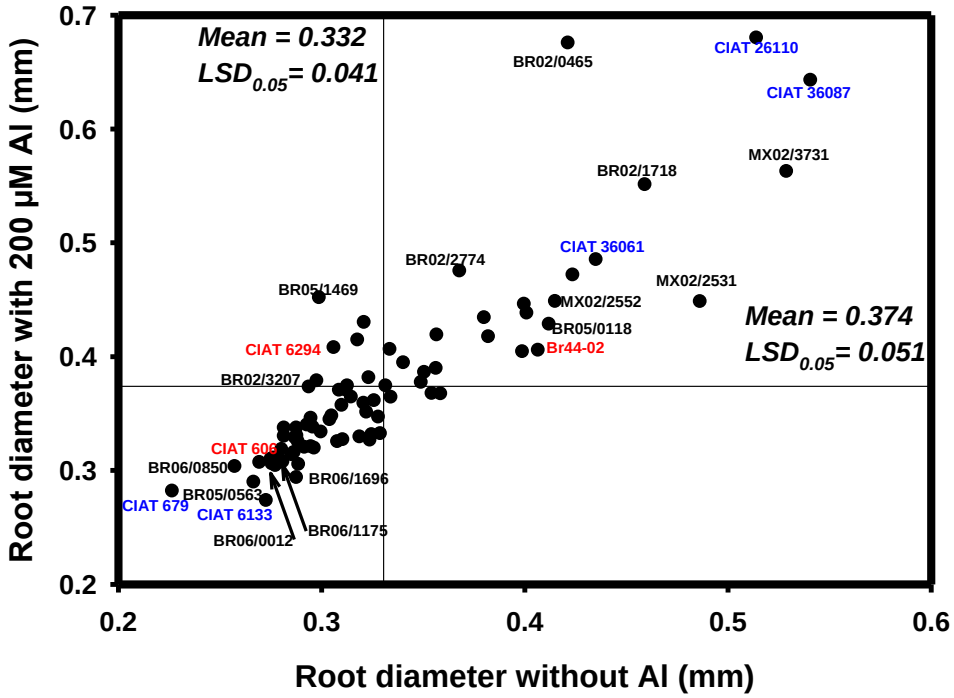


Figure 6. Relationship between mean root diameter with Al and mean root diameter without Al in solution of 79 *Brachiaria* gentoypes. Gentoypes that developed finer roots under both conditions were identified in the lower, left hand quadrant.

Total root length of the 43 *Brachiaria* genotypes was markedly decreased with Al (Table 3, Figure 7). The mean root length was 317 cm plant^{-1} under without Al treatment and this value decreased to 137 cm plant^{-1} with Al treatment showing a reduction of 57%. The mean root diameter increased from 0.381 mm to 0.487 mm (28%) with exposure to Al (Table 3, Figure 8).Under high Al in solution, 80% (24 clones) of the sexual genotypes generated more root length than the sexual parent (*B.ruziziensis* 44-02). A total of 7 clones (SX05NO/1955, SX05NO/1962, SX05NO/1948, SX05NO/2105, SX05NO/2207, SX05NO/2234 and SX05NO/2206) were found to be outstanding in root length with high Al (Table 3, Figure 7). Six sexual genotypes had finer root system (SX05NO/2234, SX05NO/1962, SX05NO/2105, SX05NO/1955, SX05NO/1948 and SX05NO/2206) with high Al in solution (Table 3, Figure 8).

Table 3. Root length and mean root diameter of 43 *Brachiaria* genotypes evaluated with (200 µM Al) and without Al (0 µM Al) in solution.

Genotypes	**Root length (cm plant^{-1})**		**Average diameter (mm)**	
	0 Al	**200 µMAl**	**0 Al**	**200 µMAl**
BRO2 NO 1372	805	410	0.2847	0.3229
Bdec CIAT 606	589	376	0.2705	0.3213
CIAT 36061	545	231	0.4256	0.5432
BRO2 NO 1752	562	195	0.3890	0.4582
Bhum CIAT 679	471	173	0.2428	0.2954
BRO2 NO 1245	402	167	0.3098	0.4442
Bdic CIAT 6133	207	166	0.2626	0.2833
SX05 NO 1955	341	164	0.3544	0.4385
SX05 NO 1962	354	162	0.3140	0.3677
SX05 NO 1948	313	155	0.3739	0.4716
SX05 NO 2105	339	152	0.3380	0.4098
SX05 NO 2207	489	151	0.3994	0.5102
SX05 NO 2234	177	148	0.3443	0.2847
Bbriz CIAT 6294	410	146	0.3516	0.5303
SX05 NO 2206	338	141	0.3619	0.4724
SX05 NO 1963	446	132	0.3526	0.4505
SX05 NO 2440	269	132	0.3708	0.5076
SX05 NO 2108	239	127	0.4103	0.5123
SX05 NO 2446	286	125	0.3715	0.4697
SX05 NO 1905	252	121	0.4411	0.5166
SX05 NO 2031	224	119	0.3769	0.4348
SX05 NO 1949	328	118	0.3796	0.5142
SX05 NO 2015	235	111	0.4656	0.5760
SX05 NO 2000	240	100	0.3802	0.5534
SX05 NO 2155	118	98	0.3467	0.3547
SX05 NO 2343	432	91	0.3344	0.4910
SX05 NO 2560	256	90	0.3748	0.4670
SX05 NO 1990	247	88	0.3568	0.4149
SX05 NO 1907	176	86	0.3748	0.4593
SX05 NO 2480	243	85	0.4329	0.5295
SX05 NO 2421	183	81	0.4965	0.6427
CIAT 36087	212	78	0.5715	0.7922
SX05 NO 2180	114	77	0.4863	0.6052
SX05 NO 2008	185	76	0.3889	0.4783
BRO2 NO 1485	170	74	0.4173	0.4919
Bruz 44-02	202	72	0.4362	0.5054

Table 3. Root length and mean root diameter of 43 *Brachiaria* genotypes evaluated with (200 µM Al) and without Al (0 µM Al) in solution.

Genotypes	Root length (cm plant^{-1})		Average diameter (mm)	
SX05 NO 1985	174	68	0.3973	0.5641
SX05 NO 2045	155	65	0.4224	0.6095
SX05 NO 1964	281	63	0.3638	0.5330
SX05 NO 2280	139	57	0.4808	0.5927
SX05 NO 1988	196	54	0.3610	0.4737
Bbriz CIAT 26110	154	50	0.4936	0.7166
SX05 NO 2010	156	49	0.3940	0.5187
Mean	**317**	**137**	**0.3811**	**0.4873**
LSD$_{0.05}$	***122***	***62***	***0.0474***	***0.0837***

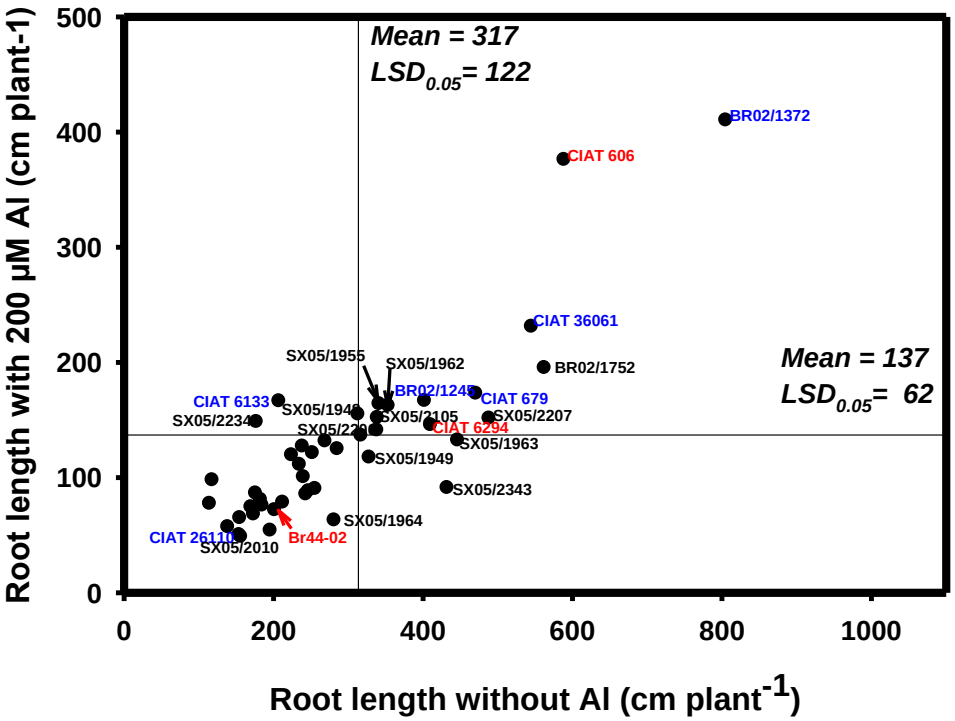


Figure 7. Relationship between total root length with Al and total root length without Al in solution of 31 *Brachiaria* gentoypes SX05N0/ and 12 checks. Gentoypes that developed greater root length under both conditions were identified in the upper, right hand quadrant.

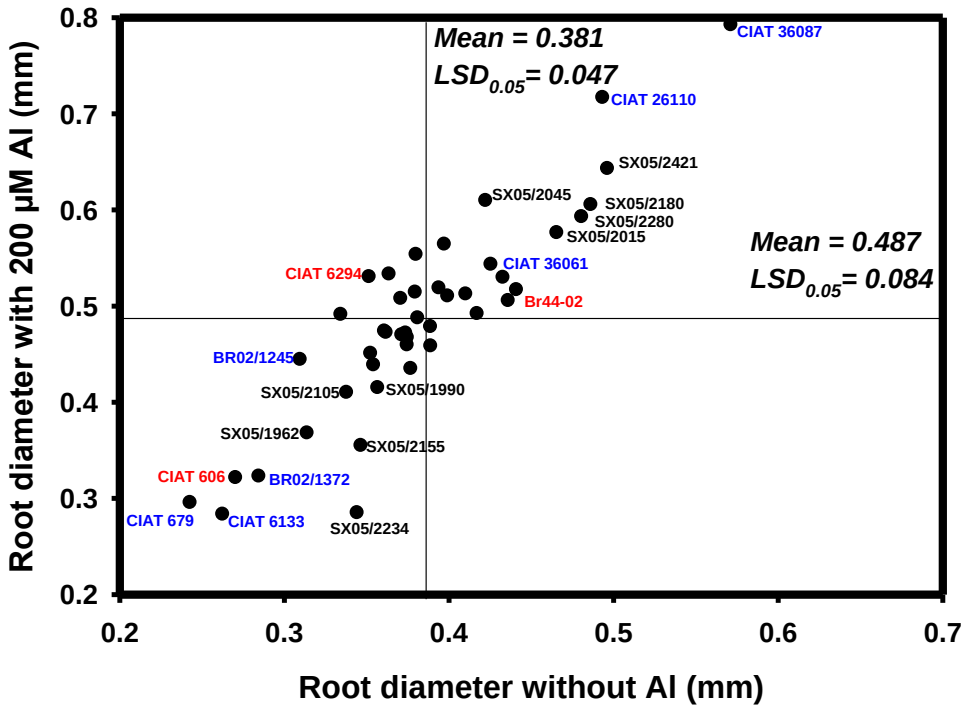


Figure 8. Relationship between mean root diameter with Al and mean root diameter without Al in solution of 31 *Brachiaria* gentoypes SX05N0/ and 12 checks. Gentoypes that developed finer roots under both conditions were identified in the lower, left hand quadrant.

Correlation coeficients between total root length and other root attributes of three groups of *Brachiaria* genotypes evaluated with 0 and 200 µM Al solution are shown in Table 4. Significant negative correlation was observed between total root length and mean root diameter for all three groups of genotypes under both with and without Al indicating that the genotypes that were resistant to Al produced much finer roots. Significan positive association was observed between total root length and root volume or surface area under both with and without Al in solution for all three groups of genotypes indicating the importance of root vigor for Al resistance. Results from these three groups of genotypes indicate the progress made so far in the breeding program in developing Al resistant apomictic and sexual hybrids of *Brachiaria*. It is important to note that some clones were markedly superior to *B. decumbens* CIAT 606 in terms of both root vogor and Al resistance.

Conclusions

Significant progress has been made in identifying both apomictic and sexual hybrids of *Brachiaria* with greater level of Al resistance. A group of nine sexual (SX05NO/1955, SX05NO/1962, SX05NO/1948, SX05NO/2105, SX05NO/2207, SX05NO/2234, SX05NO/2206, SX05NO/1953 and SX03NO/0846) and six apomictic (BR06NO/0531, BR06NO/1278, BR06NO/0012, BR06NO/1175, BR05NO/0334 and BR05NO/0537) genotypes were identified with greater level of Al resistance compared with the respective parents. Both sexual and apomictic genotypes have been improved on root development under very high aluminum levels in solution.

Table 4. Correlation coeficients between total root length and other root attributes of three groups of *Brachiaria* genotypes evaluated with 0 and 200 µM Al solution under glasshouse conditions at CIAT-Palmira.

Group of genotypes	Root attributes	Root length (m plant^{-1})	
		0 µM Al	200 µM Al
31	Root diameter (mm)	-0.49**	-0.64**
	Root volume (cm^3 plant^{-1})	0.62**	0.60**
	Surface area (cm^2 plant^{-1})	0.91**	0.93**
	Specific root length (m g^{-1})	0.354**	0.41**
79	Root diameter (mm)	-0.32**	-0.47**
	Root volume (cm^3 plant^{-1})	0.68**	0.71**
	Surface area (cm^2 plant^{-1})	0.92**	0.94**
43	Root diameter (mm)	-0.42**	-0.48**
	Root volume (cm^3 plant^{-1})	0.73**	0.57**
	Surface area (cm^2 plant^{-1})	0.93**	0.91**

** significant at the probability level of 0.01

2. Differences in shoot and root attributes of 12 *Brachiaria* genotypes subjected to aluminum toxic soil conditions

Contributors: V. Hoyos, J. Polania, J. Miles and I. Rao

Rationale: The most prominent symptom of aluminum toxicity is the inhibition of root growth. Recently, we developed a soil tube method to quantify genotypic differences in root growth and development under individual and combined stress factors of aluminum toxicity and drought stress. Last year, we determined differences in shoot and root growth responses among 12 *Brachiaria* genotypes that are subjected to three watering regimes for a period of 21 days using the soil tube method under greenhouse conditions. We found that *Brachiaria decumbens* CIAT 606 is well adapted to both intermittent and terminal drought stress conditions. Among the *Brachiaria* hybrids tested, Mulato CIAT 36061 performed better under both intermittent and terminal drought stress. The superior performance of *B. decumbens* under drought stress was associated with greater production of roots in subsoil layers. The superior performance of Mulato CIAT 36061 was associated with greater ability for leaf expansion under drought stress conditions. Among the 12 genotypes tested, the sexual hybrid SX03/0881 was least adapted to drought stress conditions. As part of a BMZ funded project, we used this greenhouse soil tube method to evaluate the shoot and root growth responses of 12 genotypes of *Brachiaria* that were subjected to Al-toxic soil conditions.

Materials and methods: A greenhouse experiment was conducted to determine differences in shoot and root attributes among 12 *Brachiaria* genotypes (3 parents: *Brachiaria decumbens* CIAT 606, *Brachiaria ruziziensis* 44-02, *Brachiaria brizantha* CIAT 6294 cv. Marandú; 2 commercial hybrids: Mulato CIAT 36061 and Mulato II CIAT 36087; 4 apomictic hybrids: BR02-1372, BR02-1752, BR02-0465 and BR02-1485; and 3 sexual hybrids: SX03-0881, SX03-0846 y SX03-2367) as affected by Al toxicity in soil (Figure 1). Plants were sown in 80 cm plastic cylinders using one stolon per tube containing 5.5 kg of soil from Matazul farm in the Llanos of Colombia (with 78.2% Al saturation) with a bulk density of 1.33 g cm^{-1}.

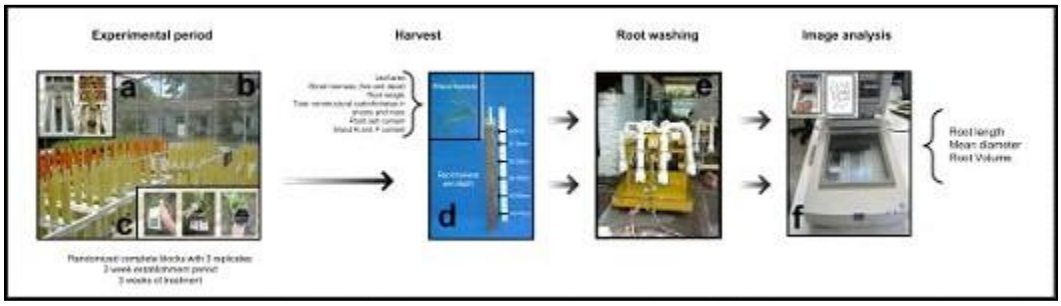


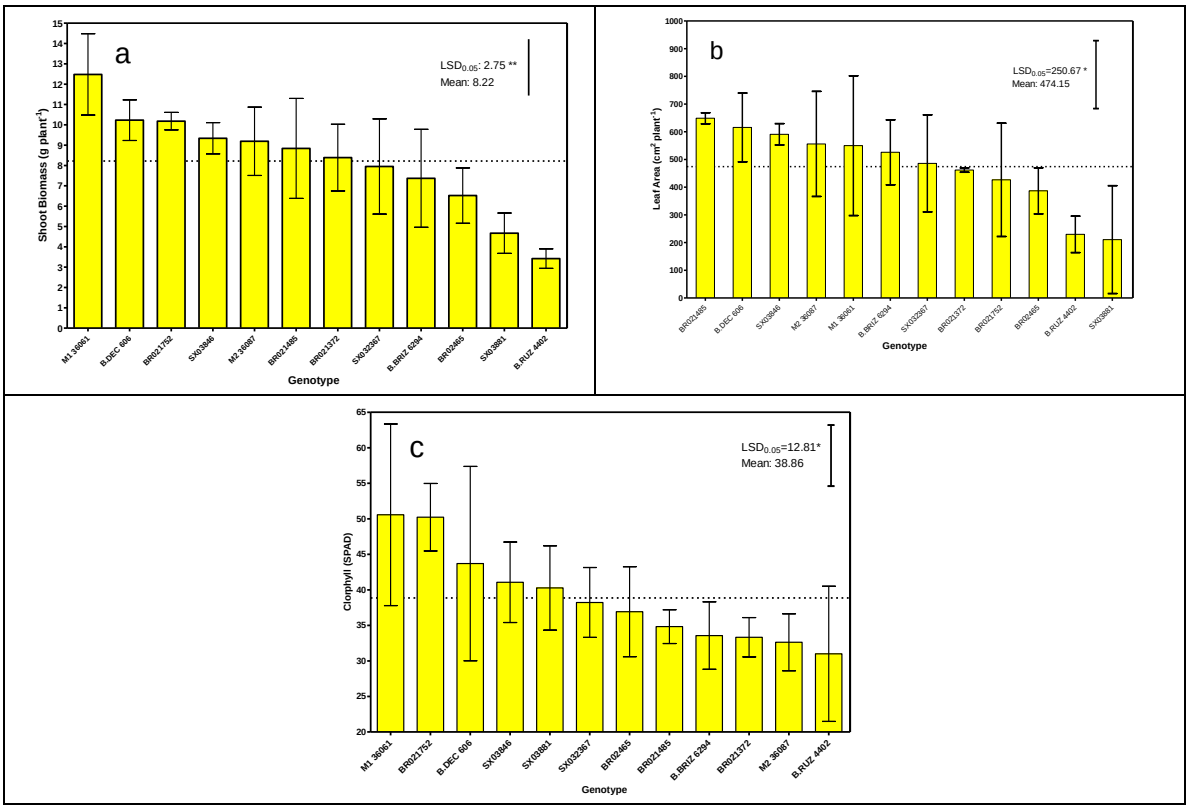
Figure 1 Methodology for screening *Brachiaria* genotypes, a) plastic tubes were covered with PVC pipes to protect from higher temperature, b) final arrangement of the experiment, c) chlorophyll, rate of transpiration, stomatal conductance and leaf temperature were measured at weekly intervals for 21 days, d) root samples collected from different soil depths, (e) root samples were washed free of soil using a hydropneumatic elutriation system, and f) root samples were analyzed using a flatbed scanner and WinRhizo to quantify root traits (root length, mean root diameter, root volume). Root biomass and specific root length were also determined after drying the root samples in the oven at 70ºC for 2 days.

Transparent plastic tubes with soil were covered with PVC tubes to minimize temperature shifts. The soil was fertilized with adequate levels of nutrients (kg/ha: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients). Plants were kept at 100% field capacity (FC). At harvest (after 3 weeks of treatment), shoot variables such as biomass (stems and leaves, live and dead), leaf area, and total nonstructural carbohydrate (TNC), ash, nitrogen and phosphorous contents in leaves and stems were determined. Physiological traits such as chlorophyll content, rate of transpiration and stomatal conductance were also determined.

For determining the root traits, the cylinders were cut into 0-5, 5-10, 10-20, 20-40, 40-60, 60-80 cm soil depths in order to determine root length, mean root diameter and root volume. Total nonstructural carbohydrate contents of roots were determined for 0-20 and 20-40 cm soil depth. The root samples from different soil depths were washed free of soil using a hydropneumatic elutriation system (Gillison's Variety Fabrication, Benzonia, Michigan, USA). The results were analyzed using the GLM procedure and LSD test of SAS v.9 for Windows, Pearson's correlation coefficients served as a tool to screen variables for higher association with shoot biomass. Also, rooting depth was determined using the cumulative root length fraction with the following model:

$Y=1-\beta d$, where Y is the cumulative root fraction from the surface of the soil, d is soil depth in cm and β is the estimated parameter. Since β is the only parameter estimated in the model, it was used to measure vertical root distribution. Higher values of β are associated with a greater proportion of roots at greater depths in relation to lower values of β, which are associated with a greater proportion of roots near the surface of the soil.

Results and Discussion: Shoot biomass presented highly significant differences ($p<0.01$) at the genotype level (Figure 2a). The genotypes with higher levels of shoot biomass production in Al-toxic soil conditions were Mulato CIAT 36061, *B. decumbens* CIAT 606, BR02-1752 and SX03-0846 while BR02-0465, SX03-0881 and *B. ruziziensis* 44-03 showed lower values. Similar to shoot biomass production, leaf area values also presented significant differences ($p<0.05$) at the genotype level. Genotypes with the highest values were BR02-1485, *Brachiaria decumbens* CIAT 606 and SX03-0846 and those with the lowest values were *Brachiaria ruziziensis* 44-02 and the sexual hybrid SX03-0881 (Figure 2b). Leaf chlorophyll content (SPAD) showed significant genotypic differences (Figure 2c).



**Significant at probability level of 0.01, * Significant at probability level of 0.05, N.S. = not significant. The dotted line is the mean value of the 12 genotypes

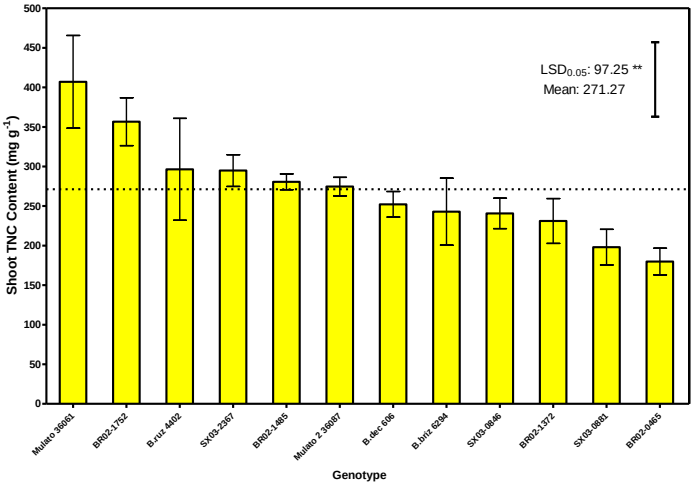
Figure 2. Differences in (a) shoot biomass, (b) leaf area and (c) chlorphyll content of 12 *Brachiaria* genotypes subjected to Al toxicity conditions for 21 days.

The genotypes that showed higher amounts of chlorophyll content were Mulato CIAT 36061, BR02-1752 and *Brachiaria decumbens* CIAT 606. Other genotypes such as BR02-1485, *Brachiaria brizantha* CIAT 6294 cv. Marandu, BR02-1372, Mulato II CIAT 36087 and *Brachiaria ruziziensis* 44-02 showed lower values. Correlation coefficients (r) between shoot biomass and a number of shoot traits are shown in Table 1. Leaf area, leaf chlorophyll content and TNC contents of leaves and stems showed positive association with shoot biomass indicating that the genotypes that were able to grow were productive under Al toxicity conditions. Leaf and stem nutrient (N and P) contents showed negative association with shoot biomass indicating that high nutrient use efficiency contributed to superior shoot biomass production. Leaf ash content also showed negative association with shoot biomass indicating the contribution of high nutrient use efficiency to leaf growth. The content of TNC in the shoots presented significant differences between genotypes (Figure 3). Genotypes such as Mulato CIAT 36061 and BR02NO-1752 showed greater values while SX03-0881 and BR02-0465 showed lower values.

Table 1. Correlation coefficients (r) between shoot biomass and other shoot traits.

Shoot traits	r
Leaf area(cm^2)	0.614**
Leaf chlorophyll content (SPAD)	0.391*
Total nonstructural carbohydrates in leaves (mg/g)	0.437**
Total nonstructural carbohydrates in stems (mg/g)	0.599**
Leaf ash content (%)	-0.466**
Stem ash content (%)	-0.088
Leaf N (%)	-0.751**
Stem N (%)	-0.558**
Leaf P (%)	-0.582**
Stem P(%)	-0.792**

(*) Significant at the 0.05 level; (**); Significant at the 0.01 level.

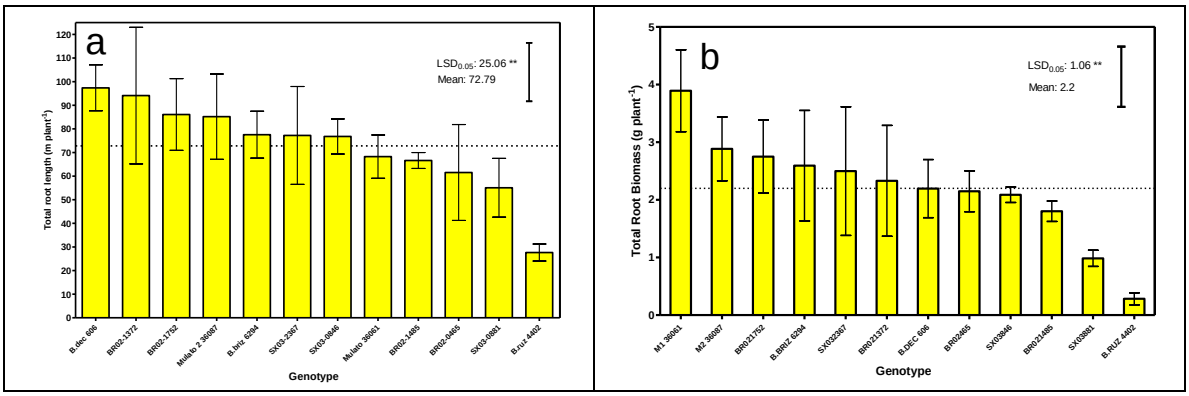


**: Significant at the 0.01 probability level, *: Significative at the 0.05 probability level. The straight vertical lines indicate LSD values at the 0.05 probability level. Dotted horizonal line indicates the genotypic mean.

Figure 3. Differences in total nonstructural carbohydrates (TNC) in the shoots of 12 *Brachiaria* genotypes subjected to Al-toxic conditions for 21 days.

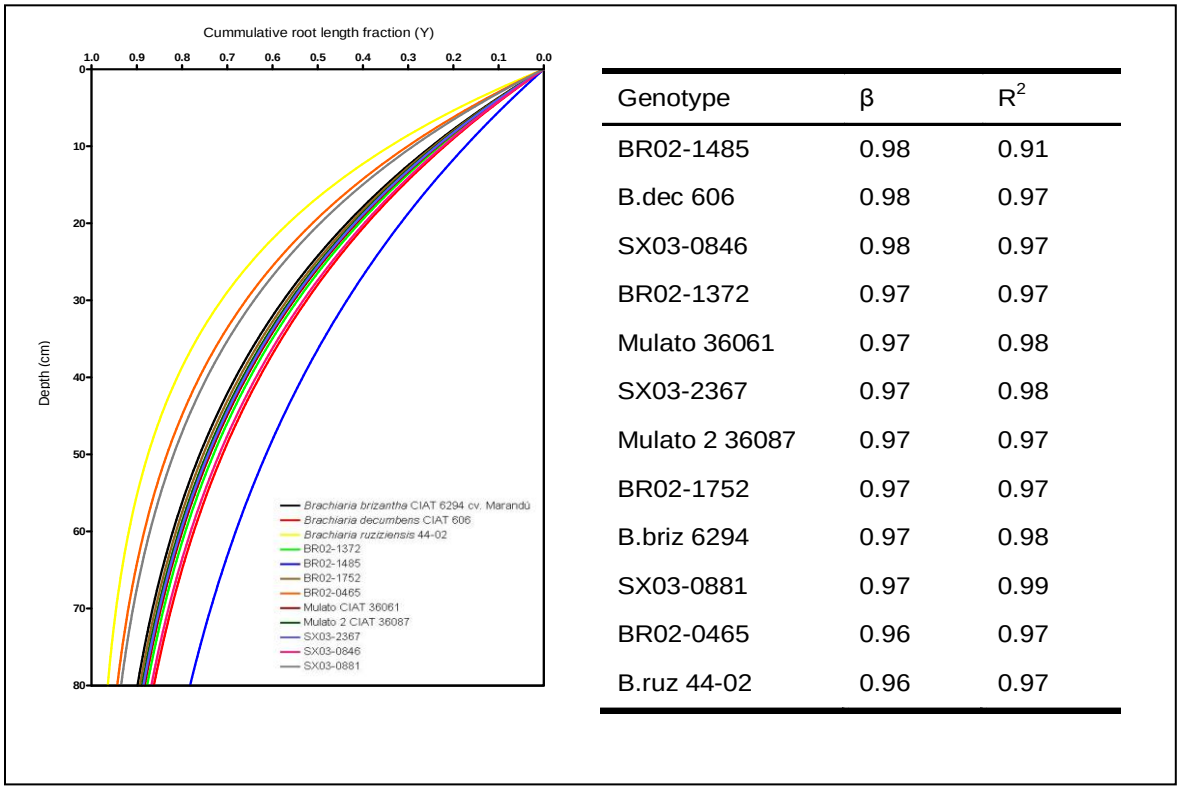
Figure 4a shows differences in total root length production of 12 *Brachiaria* genotypes. This variable showed highly significant differences ($p<0.01$) between genotypes. As expected, *B. decumbens* was outstanding in its root length production while *B. ruziziensis* was least productive in root system development. Root biomass production of cv. Mulato was outstanding while *B. ruziziensis* showed the lowest value (Figure 4b).

Results on root length distribution indicated that genotypes such as BR02-1485, *Brachiaria decumbens* CIAT 606 and SX03-0846, with β values of 0.98, had deeper root systems than genotypes such as BR02-0465 and *Brachiaria ruziziensis* 44-02, with β values of 0.96 (Figure 5).



**Significant at probability level of 0.01, * Significant at probability level of 0.05. The dotted line is the mean value of the 12 genotypes.

Figure 4. Total root length (a) and total root biomass (b) production of 12 *Brachiaria* genotypes that were subjected to Al toxicity conditions in soil for 21 days.



Genotype	β	R^2
BR02-1485	0.98	0.91
B.dec 606	0.98	0.97
SX03-0846	0.98	0.97
BR02-1372	0.97	0.97
Mulato 36061	0.97	0.98
SX03-2367	0.97	0.98
Mulato 2 36087	0.97	0.97
BR02-1752	0.97	0.97
B.briz 6294	0.97	0.98
SX03-0881	0.97	0.99
BR02-0465	0.96	0.97
B.ruz 44-02	0.96	0.97

Figure 5. Cumulative root fraction for 12 *Brachiaria* genotypes grown in Al toxic soil. Genotypes with higher or lower values of β present a deep or shallow root system, respectively

Conclusions: Among the 12 Brachiaria genotypes evaluated for root development under Al toxic soil conditions, *Brachiaria decumbens* was found to be outstanding in developing fine root system while the *Brachiaria* hybrid cv. Mulato was superior in shoot biomass production. The thicker root system of cv. Mulato was associated with its superior ability to produce shoot biomass. This study confirmed the sensitivity of *Brachiaria ruziziensis* to Al toxic soil conditions and this was attributed to its poor root system development.

3. Phenotypic differences in adaptation to drought stress in *Brachiaria* grasses

Contributors: V. Hoyos, J. Polania, J. Miles and I. Rao

Rationale: Water stress results in a decrease in cell volume, an increase in the concentration of cellular sap and the progressive dehydration of the protoplasm. There is no living process that is unaffected by a decrease in water potential. The first response to water deficits is a restriction in leaf expansion that results in reduced leaf growth under water stress. Total biomass as well as the partitioning of biomass between the shoot and the roots is influenced by the plant water status. In most cases, when water is limiting, an increase in root production relative to the shoot has been observed. There is very limited knowledge on the physiological and biochemical bases of adaptation of *Brachiaria* grasses to drought. Seasonal drought affects both quantity and quality of forage in tropical subhumid environments. *Brachiaria* grasses differ in drought resistance. *B. brizantha* CIAT 6780, *B. decumbens* CIAT 606, Mulato and Mulato II are known to be relatively more adapted to drought stress. Our objective was to determine differences in shoot and root growth responses among 12 *Brachiaria* genotypes that are subjected to three watering regimes for a period of 21 days using the soil tube method under greenhouse conditions.

Materials and methods: A greenhouse experiment was conducted to determine differences in shoot and root attributes of 12 *Brachiaria* genotypes (3 parents of the breeding program: *Brachiaria decumbens* CIAT 606, *Brachiaria ruziziensis* 44-02, and *Brachiaria brizantha* CIAT 6294 cv. Marandú; 2 commercial hybrids: Mulato CIAT 36061 and Mulato II CIAT 36087; 4 apomictic hybrids: BR02/1372, BR02/1752, BR02/0465 and BR02/1485; and 3 sexual hybrids: SX03/0881, SX03/0846 and SX03/2367) that were subjected to drought conditions for 21 days.

The experimental system to evaluate the response to drought stress is shown in Figure 1. Plants were sown in 80 cm plastic tubes using one stolon per soil tube containing 5.5 kg of Matazul soil mixed with sand in a 2:1 ratio with a final bulk density of 1.33 g cm-3. The soil was fertilized with adequate levels of nutrients (kg/ha: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients). These plastic soil tubes were inserted into PVC tubes to decrease higher temperature effects in the greenhouse. The average maximum and minimum temperature values were 35ºC and 21ºC, respectively with a maximum photon flux density of 1000 µmol m-2 s-1. Three watering levels were maintained: 100% field capacity (FC) as control or well watered, 50% FC to simulate intermittent drought and terminal drought by withholding water after establishment. The treatments of 100% FC and 50% FC were kept at their respective levels by weighing the soil tubes at every 2 days and applying water to the surface of the soil. Terminal drought treatment was imposed at three weeks after planting. The experiment comprised a completely randomized block design with 3 replicates (watering levels as main plots and genotypes as subplots).

At harvest, shoot traits such as leaf biomass, stem biomass, leaf area, and total nonstructural carbohydrates, ash, nitrogen and phosphorous contents in leaves and stems were determined. Other shoot traits such as leaf chlorophyll content, rate of transpiration and stomatal conductance were also determined at weekly intervals during the stress treatment. For the root traits, at the time of harvest, the soil tubes were cut into 0-5, 5-10, 10-20, 20-40, 40-60, 60-75 cm soil depths in order to determine root length, mean root diameter and root volume across soil depth and total nonstructural carbohydrates at the 0-20 and 20-40 cm soil depth. These samples were washed free of soil using a hydropneumatic elutriation system (Gillison's Variety Fabrication, Benzonia, Michigan, USA). Rooting depth was determined using the cumulative root length fraction with the following model: $Y=1-\beta d$. Where Y is the cumulative root fraction from the surface of the soil d in cm and β is the estimated parameter, since β is the only parameter estimated in the model, it was used to measure vertical root distribution. Higher values of β are associated with a greater proportion of roots at greater depths in relation to lower values of β, which are associated with a greater proportion of roots near the surface of the soil. The results were analyzed using the GLM procedure and LSD test of SAS v.9 for Windows. Pearson correlation coefficients served as a tool to screen variables for higher association with shoot biomass.

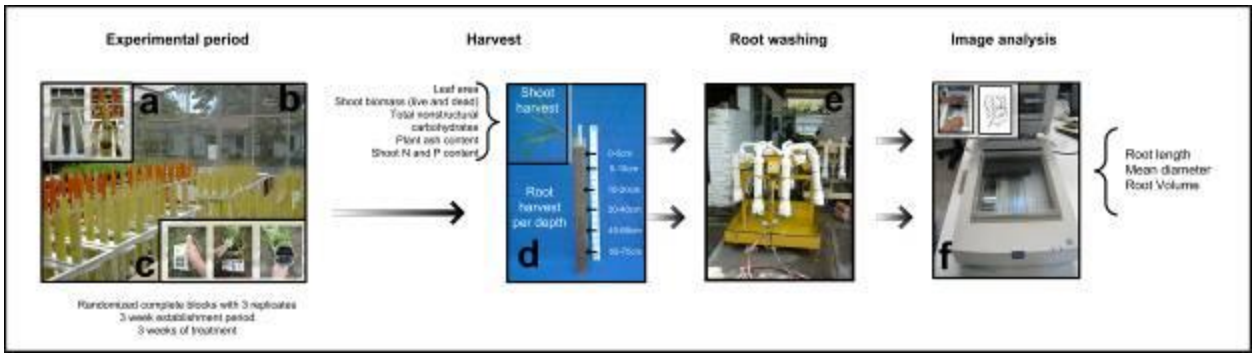


Figure 1. Methodology for screening Brachiaria genotypes, a) plastic tubes were covered with PVC pipes to protect from higher temperature, b) final arrangement of the experiment, c) chlorophyll, rate of transpiration, stomatal conductance and leaf temperature were measured at weekly intervals for 21 days, d) root samples collected from different soil depths, (e) root samples were washed free of soil using a hydropneumatic elutriation system, and f) root samples were analyzed using a flatbed scanner and WinRhizo to quantify root traits (root length, mean root diameter, root volume). Root biomass and specific root length were also determined after drying the root samples in the oven at 70ºC for 2 days.

Results and discussion: Highly significant differences were found among genotypes and treatments ($p<0.01$) on live shoot biomass production (Figure 2). However, there was no effect on genotype x watering level interaction in shoot biomass production. Genotypic differences in shoot biomass were nonsignificant for well watered or control treatment but were significant for terminal and intermittent drought treatments. It is important to note that adequate amount of nutrients were supplied in the system. It is known that under lower nutrient supply *B. decumbens* CIAT 606 performs better than *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02. As expected, drought reduced the genotypic mean values of shoot biomass by 39% and 70% for intermittent and terminal drought, respectively. Among the 12 genotypes tested, *Brachiaria decumbens* CIAT 606 performed better under water stress conditions while the sexual hybrid SX03/0881 was the poor performer in terms of shoot biomass production. Among the hybrids, Mulato CIAT 36061 performed better under both intermittent and terminal drought stress. The sexual parent *Brachiaria ruziziensis* 44-02 was outstanding in producing shoot biomass under control treatment but its shoot growth was markedly affected by drought stress, particularly with terminal drought stress (Figure 2). This genotype requires adequate nutrient supply to perform better under well watered conditions.

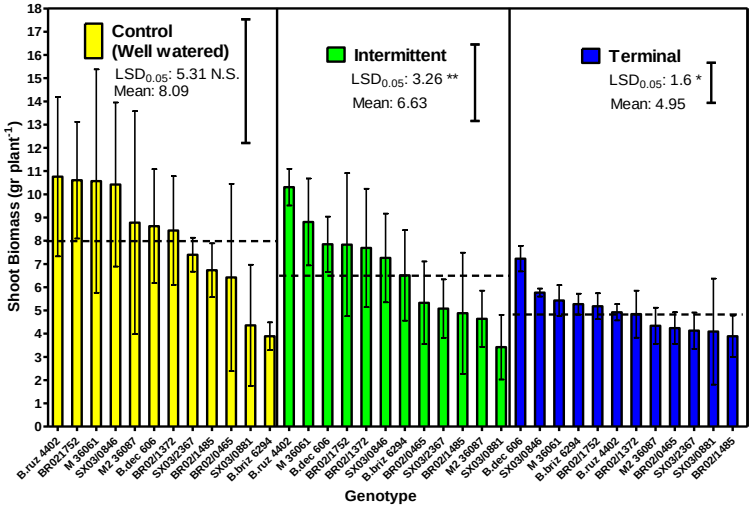


Figure 2. Live shoot biomass production of 12 Brachiaria genotypes grown under control (well watered), intermittent and terminal drought stress conditions. M = Mulato, M2 = Mulato II. **, *: Significant at the 0.01 and 0.05 probability level, respectively. N.S.= not significant. The bars indicate LSD values at the 0.05 probability level. Dotted horizontal lines indicate the mean values.

The relationship between live shoot biomass production and other plant attributes is shown in Table 1. While the shoot biomass production was significantly related to leaf area production across all three treatments, the relationship was stronger with well watered conditions than the drought treatments. The dead leaf biomass was in terminal drought stress was positively associated with shoot biomass production indicating the importance of internal mobilization of photosynthates and nutrients to the growth of green leaves. Leaf chlorophyll content was positively associated with shoot biomass production under intermittent drought stress and this could be due to new leaf growth. Under terminal drought stress, leaf chlorophyll content was negatively associated with shoot biomass indicating the importance of remobilization of photosynthates and N. The rate of transpiration at 18 days after terminal drought stress showed negative relationship with green leaf area production indicating the importance of stomatal regulation for improving water use efficiency (Figure 3). Genotypes that combined higher values of green leaf area with lower values of the rate of transpiration are shown in lower right quadrant. These included *Brachiaria decumbens* CIAT 606, BR02/1752, BR02/0465 Mulato CIAT 36061 and *Brachiaria brizantha* CIAT 6294 cv. Marandú that could be more efficient in using water for producing green leaf area under terminal drought stress. Among the hybrids tested, the sexual hybrid SX03/0881 showed lower value of green leaf area and moderately high value of the rate of transpiration. The highest rate of transpiration was observed with Mulato II CIAT 36087. Rate of transpiration and stomatal conductance were negatively related to shoot biomass production at 18 days across treatments but the relationship was not significant (Table 1). With intermittent drought stress, the level of leaf and stem total nonstructural carbohydrates (TNC) showed significant positive association with live shoot biomass indicating greater availability of photosynthates for new shoot growth.

Table 1. Correlations (r) between live shoot biomass production and other plant attributes of 12 *Brachiaria* genotypes subjected to drought stress

Variable	Shoot biomass		
	Control	Intermittent drought	Terminal drought
Leaf area (cm^2)	0.808**	0.510**	0.342*
Dead leaf biomass(g)	0.074	-0.136	0.346*
Chlorophyll content (SPAD)	0.215	0.460**	-0.402*
Rate of transpiration (mmol H_2O m^{-2} s^{-1}) at 18 days after treatment	-0.036	-0.221	-0.311
Mean stomatal conductance (mmol H_2O m^{-2} s^{-1}) at 18 days after treatment	-0.031	-0.231	-0.280
Total nonstructural carbohydrates in leaves (mg/g)	0.274	0.600**	0.145
Total nonstructural carbohydrates in stems (mg/g)	0.430**	0.456**	0.126
Root length (10-75 cm)	0.514**	0.485**	0.209
Root diameter (0-20 cm)	0.257*	0.189	-0.116
Root volume (0-75 cm)	0.454**	0.268*	0.059

*, ** significant at the 0.05and 0.01 level, respectively.

As in shoot biomass, the genotypic mean values of total root length were reduced by both intermittent and terminal drought stress compared with control treatment (Figure 4). Among the hybrids, Mulato CIAT 36061 performed better in terms of total root length under drought stress, particularly under terminal stress. One of the sexual hybrids, SX03/881 showed the lowest value of total root length across the three treatments. One of the apomictic hybrids, BR02/1372 that was known to have higher level of Al resistance showed moderate values of total root length under both intermittent and terminal drought stress.

Differences in root length, mean root diameter, root biomass and specific root length across soil depth under different treatments for 5 contrasting Brachiaria genotypes are shown in Figure 5. Results on root length distribution across soil depth showed significant genotypic differences under terminal drought stress. Mulato CIAT 36061, *B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294 cv. Marandu showed greater values of root length and root biomass across soil depth in all three treatments. The sexual hybrid SX030881 showed lower values of both root length and root biomass distribution across soil depth. The sexual parent, *B. ruziziensis* 44-02 had greater values of root length and root biomass under well watered conditions but drought stress decreased the values, particularly under terminal drought stress.

Relationships between shoot biomass production and root attributes are shown in Table 1. Root length (10-75 cm soil depth) and root volume were positively associated with shoot biomass production under well watered and intermittent drought stress conditions. Under well watered conditions, root diameter also showed positive association with shoot biomass indicating that the thicker the root system the greater the vigor of the shoot when neither water nor nutrients were limiting in the system. Negative association was observed between root diameter and shoot biomass under terminal drought stress indicating the importance of development of finer roots in deeper soil layers.

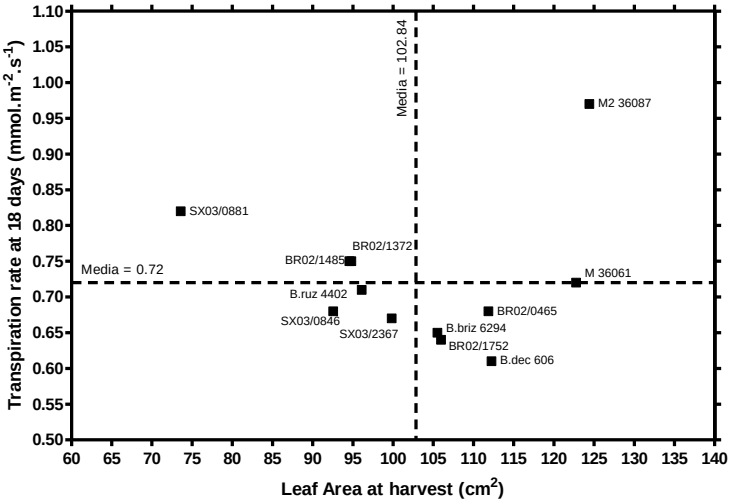


Figure 3. Relationship between leaf area at harvest and the rate of transpiration measured at 18 days of terminal drought stress for 12 *Brachiaria* genotypes. M = Mulato, M2 = Mulato II. Vertical and horizontal dotted lines represent the genotypic mean values.

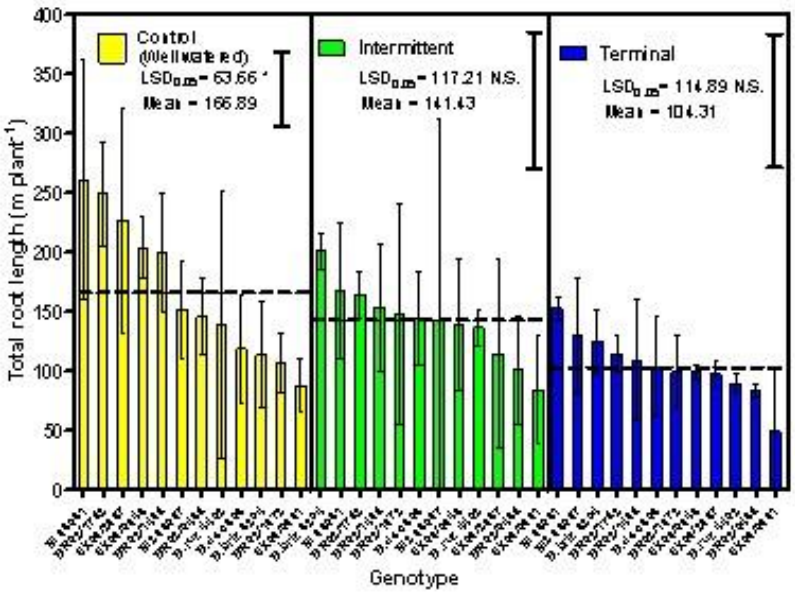


Figure 4. Total root length of 12 *Brachiaria* genotypes grown under control (well watered), intermittent and terminal drought stress conditions. M = Mulato, M2 = Mulato II. **, *: Significant at the 0.01 and 0.05 probability level, respectively. N.S.= not significant. The bars indicate LSD values at the 0.05 probability level. Dotted horizontal lines indicate the mean values.

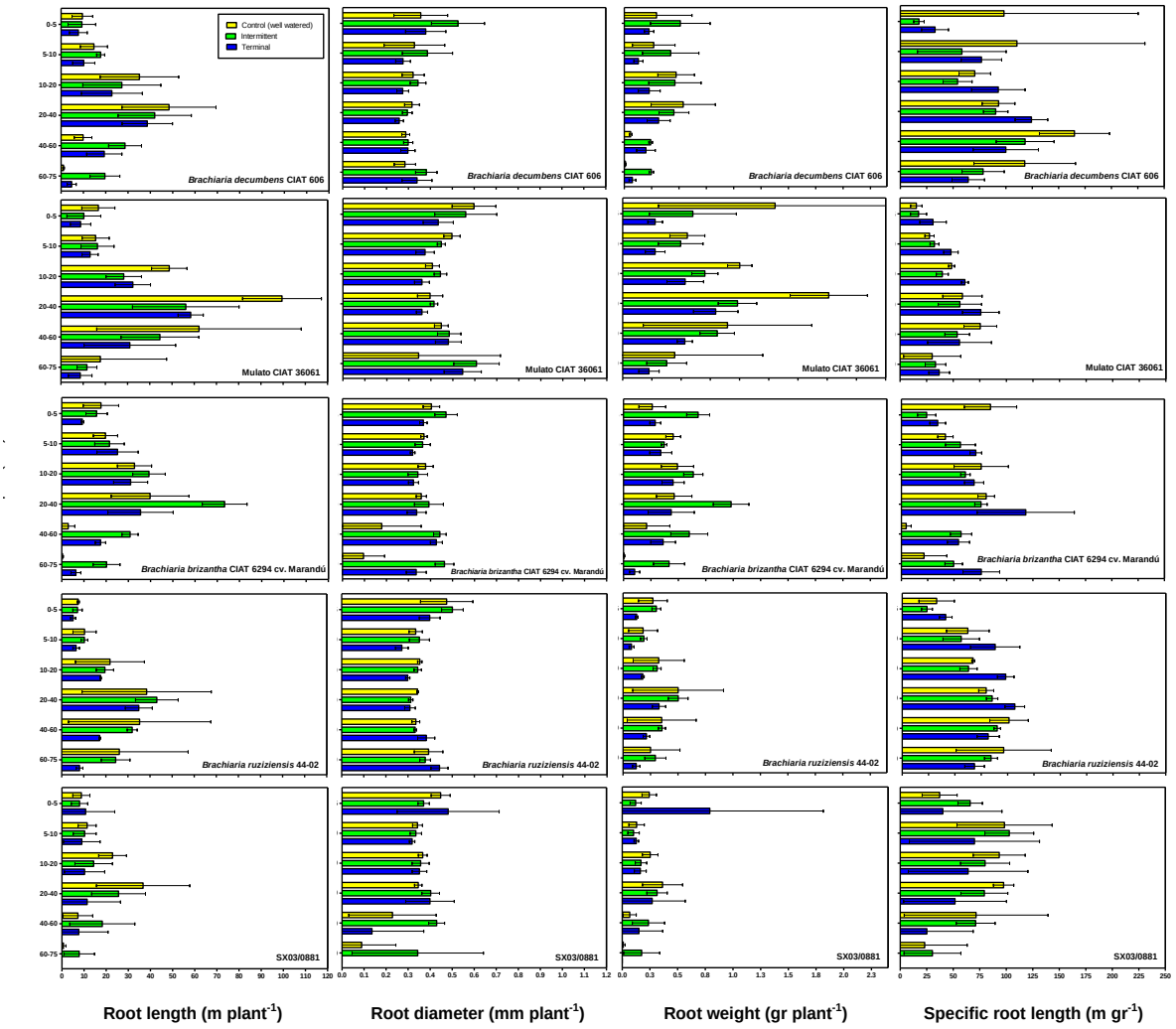
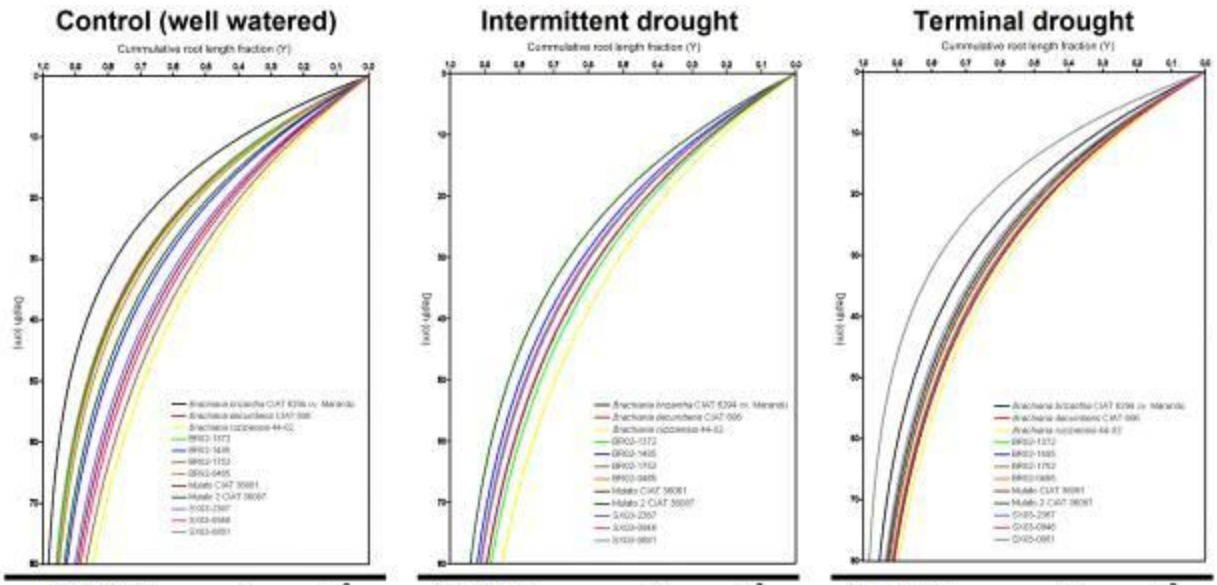


Figure 5. Differences in root length, root diameter, root biomass and specific root length across the soil profile at harvest for 5 contrasting *Brachiaria* genotypes (*Brachiaria decumbens* CIAT 606, Mulato CIAT 36061, *Brachiaria brizantha* CIAT 6294 *cv*. Marandú, *Brachiaria ruziziensis* 44-02 and SX03/0881) that were subjected to drought conditions for 21 days.

Figure 6 shows differences in vertical root length distribution (cumulative proportion) among the *Brachiaria* genotypes together with the values of β and R^2 for each of the treatments. The higher values of β indicate greater proportion of root length at deeper soil layers while the lower values indicate greater proportion of root length in surface soil layers. Among the genotypes tested, Mulato CIAT 36061 maintained its deep rooting ability across stress treatments. The sexual parent *B. ruziziensis* showed deeper rooting ability under well watered and intermittent stress conditions. Among the 12 genotypes tested, the sexual hybrid SX03/0881 showed lower value of β under terminal drought stress indicating greater proportion of root length in the top soil layers.



Genotype	β	R^2
B.ruz 44-02	0.98	0.95
BR02/1752	0.98	0.94
SX03/0846	0.97	0.95
Mulato 36061	0.97	0.95
SX03/2367	0.97	0.97
BR02-1485	0.97	0.96
Mulato 2 36087	0.97	0.95
BR02/0465	0.96	0.96
BR02/1372	0.96	0.98
B.dec 606	0.96	0.96
SX03/0881	0.96	0.96
B.briz 6294	0.95	0.98

Genotype	β	R^2
B.ruz 4402	0.98	0.95
BR02/1372	0.97	0.96
Mulato 36061	0.97	0.95
B.dec 606	0.97	0.97
BR02/1752	0.97	0.96
SX03/0881	0.97	0.98
SX03/0846	0.97	0.97
B.briz 6294	0.97	0.97
BR02/0465	0.97	0.99
SX03/2367	0.97	0.99
BR02/1485	0.97	0.97
Mulato 2 36087	0.97	0.98

Genotype	β	R^2
B.ruz 4402	0.97	0.95
Mulato 36061	0.97	0.95
SX03/0846	0.97	0.95
BR02/1372	0.97	0.96
BR02/1752	0.97	0.95
B.dec 606	0.97	0.96
BR02/1485	0.97	0.97
Mulato 2 36087	0.97	0.97
BR02/0465	0.97	0.97
SX03/2367	0.97	0.97
B.briz 6294	0.96	0.98
SX03/0881	0.95	1.00

Figure 6. Vertical root length distribution (cumulative proportion) for 12 *Brachiaria* genotypes under different drought stress treatments at the time of harvest as a function of β. Higher values of β indicate deeper root system.

Conclusions: Results from this study indicate that *Brachiaria decumbens* CIAT 606 is well adapted to both intermittent and terminal drought stress conditions. Among the *Brachiaria* hybrids tested, Mulato CIAT 36061 performed better under both intermittent and terminal drought stress. The superior performance of *B. decumbens* under drought stress was associated with greater production of roots in subsoil layers. The superior performance of Mulato CIAT 36061 was associated with greater ability for leaf expansion under drought stress conditions. Among the 12 genotypes tested, the sexual hybrid SX03/0881 was least adapted to drought stress conditions.

4. Differences in regulation of water use, water use efficiency and growth of six Brachiaria genotypes exposed to combined stress conditions of drought and aluminum toxicity

Contributors: V. Hoyos, J. Polania, J. Miles and I. Rao

Rationale: Adaptation to drought involves complex multigenic components that interact holistically in plant systems and maintaining root growth plays a key role. Soil drying decreases shoot growth rate, plant height, and yield, but affects root growth less. Water loss may be reduced by leaf morphological attributes or due to early stomatal closure in response to abscisic acid (ABA) transported in xylem from root to shoot and perceived at the guard cell apoplast. Al toxicity affects root development, which in turn affects the acquisition of nutrients and water. There is very limited knowledge on the physiological and biochemical bases of brachiariagrass' adaptation to either individual or combined stress factors of drought and Al toxicity. Seasonal drought affects both quantity and quality of forage in tropical savanna environments. *Brachiaria* grasses differ in their level of drought resistance. *B. brizantha* CIAT 6780 and *B. decumbens* CIAT 606 were found to be relatively more adapted to drought stress. One of the physiological mechanisms for improving drought resistance involves developing genotypes with high water use efficiency (WUE, the quantity of forage dry matter accumulated per unit of soil water transpired). Another physiological mechanism that also contributes to drought resistance is the decline in whole plant water use during soil water deficit. During soil water deficit, plants could undergo a transition between the water-replete phase where whole plant water use is not dependent on the soil water content and a second phase where water use is directly related to the availability of soil water. This transition is associated with a reduction in the average stomatal conductance and can occur at different soil water contents for different plant species or cultivars. Our objective was to determine the differences in regulation of water use, water use efficiency and growth among the 4 cultivars and two hybrids of *Brachiaria* that were subjected to combined stress of drought and Al toxicity. This knowledge is needed to develop effective screening method(s) to evaluate *Brachiaria* hybrids generated by the *Brachiaria* breeding program at CIAT for their level of resistance to combined stress of drought and Al toxicity.

Materials and methods: A greenhouse experiment was conducted to determine differences in regulation of water use, WUE (water use efficiency) and shoot growth of *Brachiaria*. Six genotypes (*Brachiaria decumbens* CIAT 606 cv. Basilisk, *Brachiaria ruziziensis* 44-02, *Brachiaria brizantha* CIAT 6294 cv. Marandú, *Brachiaria brizantha* CIAT 26110 cv. Toledo, *Brachiaria* hybrid Mulato (CIAT 36061) and *Brachiaria* hybrid Mulato II (CIAT 36087) that were subjected to a combined stress of drought and Al toxicity in soil. Soil from Matazul farm in the Llanos of Colombia (with 80% Al saturation) with adequate supply of nutrients (kg/ha: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients) was used to eliminate low nutrient supply effects but maintain Al toxicity effects. The experimental design used was completely randomized arrangement with 2 levels of water supply (well watered and terminal drought) and six genotypes with 4 replications. One stolon was planted per pot (3.5 kg of soil) and was well watered for 3 weeks. At the time of imposing the two treatments (100% field capacity (FC) and terminal drought), the pots were fully irrigated and allowed to drain until reaching a constant weight. The amount of water held at 100% FC is the maximum amount of water held in soil after free drainage. This weight was recorded and used to maintain 100% FC treatment. For inducing terminal drought stress, water supply was simply withheld. The surface of the pot was sealed with plastic in order to avoid evaporative water losses (Figure 1).

The weight of each pot was recorded every day in order to determine water loss due to transpiration based on weight difference between the days. At the end of the drying cycle, the transpiration data of the terminal drought pots was normalized by correcting the transpiration of the stressed plant against that of the control pots (100% FC) to obtain a transpiration ratio (TR), which helps to minimize the influence of large variations in transpiration across days, a second normalization was made using the TR of the same drought pot from the first days of the experiment to correct for any differences in plant size, this gives a normalized transpiration ratio (NTR). The experiment was completed when this value reached ~0.1 for each pot, which was defined as the endpoint. This value in terms of experiment duration (days) is variable because of the differential response of the genotypes to applied stress. Nevertheless, the last pot was harvested at 51 days after planting (21 days after establishment and 30 days after stress induction). This was the experimental timeframe.

After reaching the endpoint, the plants were harvested and the total plant (shoot + root) dry weights were recorded. This weight was corrected with the weight of the plant before inducing drought by using an additional set of 4 plants per genotype to record fresh and oven dry weights. To obtain a value for soil moisture, a fraction of transpirable soil water (FTSW) was also calculated from:

$$\text{Daily FTSW} = \frac{\text{daily pot weight - final pot weight}}{\text{initial pot weight - final pot weight}} \qquad (1)$$

The results from these two variables were fitted to the following equation to obtain a curve explaining the behavior of the plant during terminal drought stress:

$$\text{NTR} = \frac{1}{[1 + \text{A} \times \exp(\beta \times \text{FTSW})]} \qquad (2)$$

The results were analyzed using the GLM procedure and the LSD test from SAS v.9 for Windows

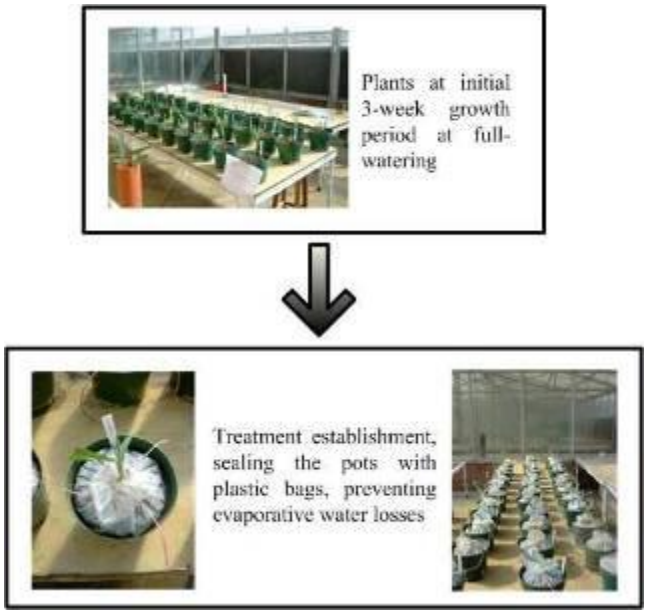


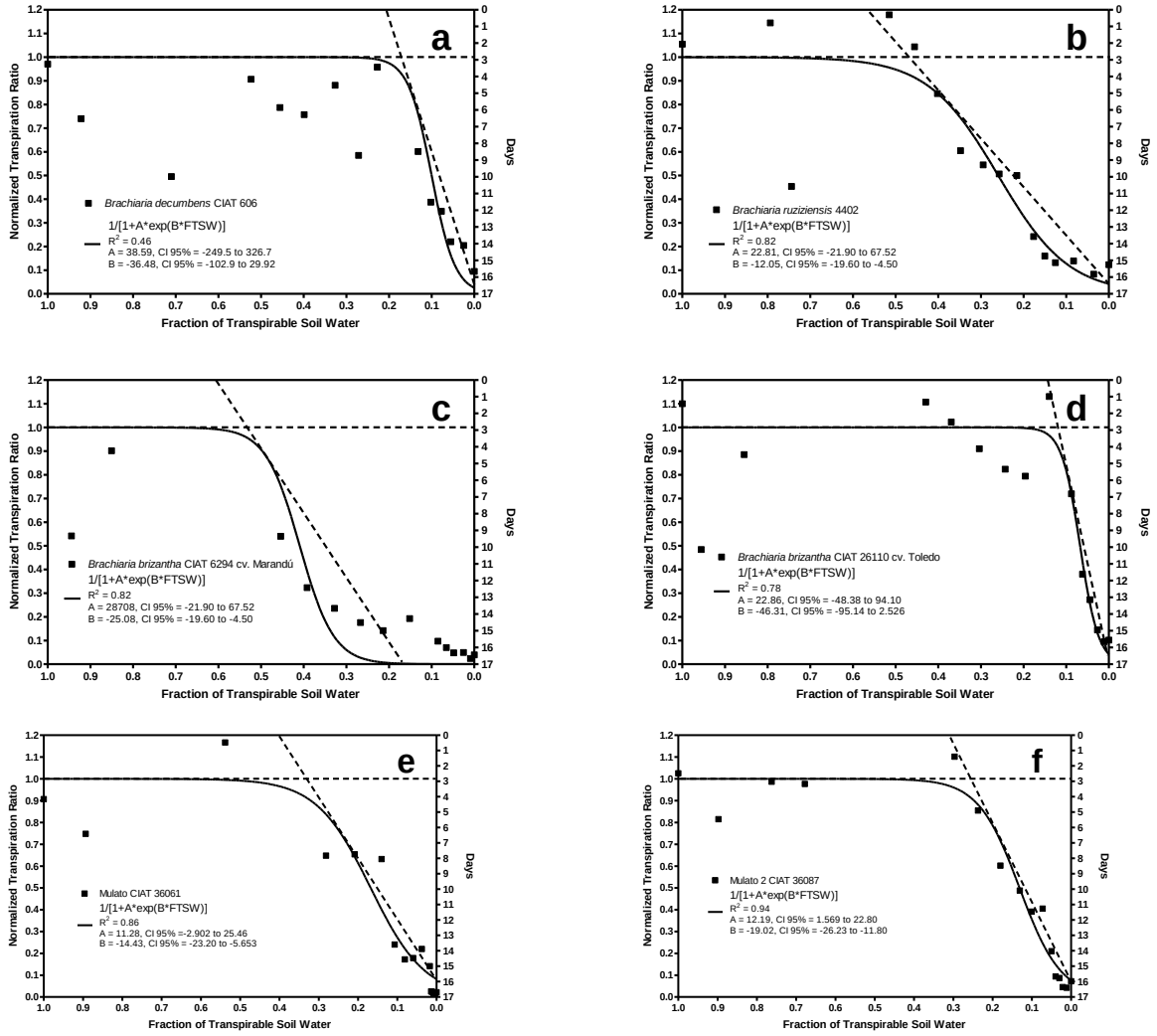
Figure 1. Methodology employed for determining FTSW (fraction of transpirable soil water) response curves.

Results: *Drought response curves:* The daily values of normalized transpiration ratio (NTR) were the basis for expressing relative transpiration rate, and the values of the fraction of transpirable soil water (FTSW) expressed the relative soil water content. Results on NTR-FTSW curve for six genotypes of *Brachiaria* during soil drying or terminal drought are shown in Figure 2.

The level of adjustment (based on determination coefficients and 95% confidence intervals) of the transpiration values to the equation mentioned above and the inflection point that occurs in the resulting curve enables to determine the point at which transpiration began to decline for each genotype. This is represented as $FTSW_c$ (critical value for the fraction of transpirable soil water at which transpiration is first reduced during a drying cycle). $FTSW_c$ is the critical soil water content at which each plant began to reduce its water use. Among the 6 genotypes tested *B. brizantha* cv. Toledo and *B. decumbens* CIAT 606 showed lower values of $FTSW_c$ indicating their superior level of adaptation to drought stress in an Al-toxic soil (Table 1).

Full stomatal closure of the genotypes was observed on a mean value of 13.5 days and a FTSW (fraction of transpirable soil water) of 30% (Table 1), with *B. decumbens* (15.25 days, Figure 2a) and *B. brizantha* CIAT 26110 cv. Toledo (14.75 days, Figure 2d) showing higher values and Mulato II CIAT 36087 (13 days, Figure 2e) and Mulato CIAT 36061 (11.5 days, Figure 2f) showing lower values. The genotypes that

showed lowest values of FTSW were *B. brizantha* CIAT 26110 cv. Toledo (14%, Figure 2d) and *B. decumbens* CIAT 606 (17%, Figure 2a), while the higher values were observed with *B.ruziziensis* 44-02 (46%, Figure 2b) and *B. brizantha* CIAT 6294 cv. Marandu (50%, Figure 2c).



a. NTR – FTSW response curve for *Brachiaria decumbens* CIAT 606 cv. Basilisk
b. NTR – FTSW response curve for *Brachiaria ruziziensis* 44-02
c. NTR – FTSW response curve for *Brachiaria brizantha* CIAT 6294 cv. Marandú
d. NTR – FTSW response curve for *Brachiaria brizantha* CIAT 26110 cv. Toledo
e. NTR – FTSW response curve for cv. Mulato CIAT 36061
f. NTR – FTSW response curve for cv. Mulato II CIAT 36087

Figure 2. NTR (normalized transpiration ratio) – FTSW (fraction of transpirable soil water) response curves of 6 *Brachiaria* genotypes during the water deficit regime. Symbols represent mean daily values (n=4). The solid line represents the fit of the data to equation 2. The intersection of the dashed lines is the point at which stomata begin to close.

Table 1. Means for the $FTSW_C$ (critical values for the fraction of transpirable soil water at which transpiration is first reduced during a drying cycle), days to endpoint and FTSW value on endpoint for 6 genotypes during drying of an Al-toxic soil.

Genotype	$FTSW_c$	Days to endpoint	FTSW on endpoint
Brachiaria brizantha CIAT 26110 cv. Toledo	0.14	14.75	0.02
Brachiaria decumbens CIAT 606	0.17	15.25	0.02
Brachiaria hybrbid cv. Mulato II CIAT 36087	0.24	13.00	0.04
Brachiaria hybrbid cv. Mulato CIAT 36061	0.28	11.50	0.07
Brachiaria ruziziensis 44-02	0.46	13.50	0.13
Brachiaria brizantha CIAT 6294 cv. Marandú	0.50	13.25	0.05
Mean	**0.30**	**13.54**	**0.06**
Significance	*	N.S.	N.S.
$LSD_{0.05}$	0.30	3.19	0.15

* Mean is significant at the 0.05 probability level. N.S.= not significant

In this sense, the combined stress conditions of terminal drought and Al stress, were physiologically more tolerated by *B.decumbens* CIAT 606 and *B. brizantha* CIAT 26110 cv. Toledo, by showing a delay in stomatal closure through an efficient use of the moisture stored in the soil during dehydration process (Figure 3). Genotypes such as *B. brizantha* CIAT 6294 cv. Marandu and *B. ruziziensis* 44-02 were found to be sensitive to combined stress conditions.

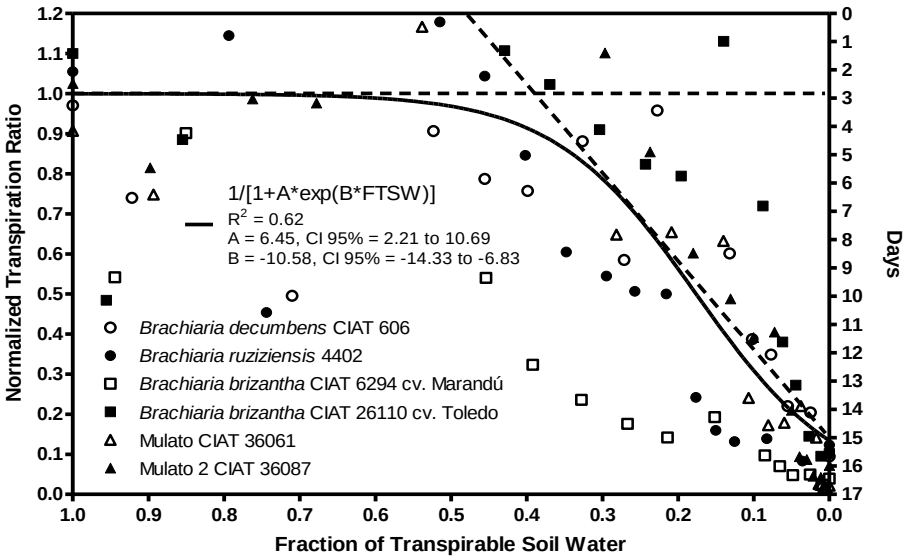


Figure 3. The relationship between the normalized transpiration ratio (NTR) and the daily values of the fraction of transpirable soil water (FTSW) for 6 genotypes of *Brachiaria* during terminal drought stress. The solid line represents the composite fit of all the data to equation 2. The intersection of the dashed lines is the point at which stomata begin to close.

In terms of water use (Figure 4), genotypes such as Mulato CIAT 36061 and Mulato II CIAT 36087 were found to be using more amount of water for shoot growth under well watered and drought stress conditions while genotypes such as *B. brizantha* CIAT 6294 cv. Marandú and *B. ruziziensis* 44-02 were using less water due to early stomatal closure. However, genotypes such as *B. decumbens* CIAT 606 and

B. brizantha CIAT 26110 cv. Toledo showed lower rates of water use with little or no change in their transpiration rates together with a delay in stomatal closure under combined stress.
Results from Figure 4 showed that during soil drying, *B. decumbens* CIAT 606 was the most "conservative" genotype in the use of water by regulating stomatal response while Mulato (CIAT 36061) and Mulato II (CIAT 36087) were the most demanding genotypes for water to maintain growth. *B. ruziziensis* 44-02 used less water due to its reduced growth due to soil drying and Al toxicity.

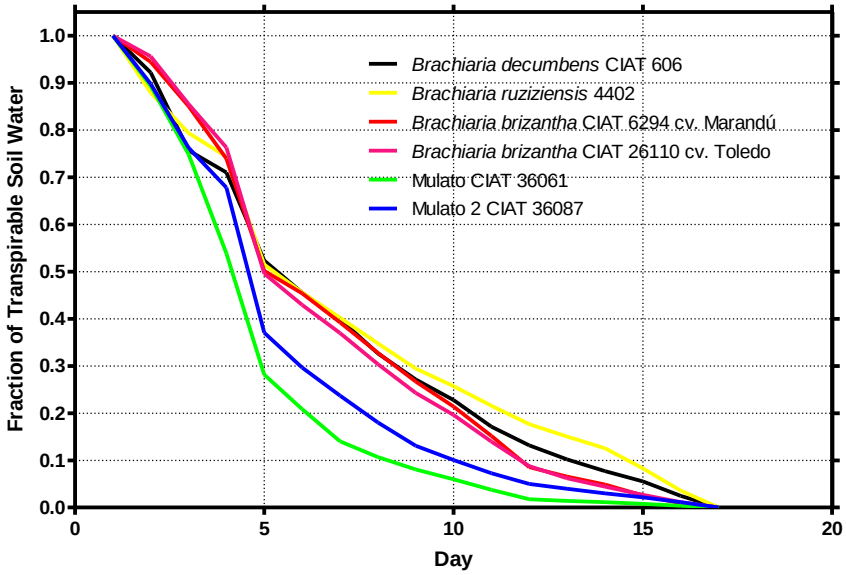


Figure 4. Differences in FTSW values over time for 6 genotypes of *Brachiaria* during soil drying of an Al-toxic soil.

In terms of water use efficiency (WUE), differences were found between 100% FC and terminal drought stress levels (Figure 5). Even though no significant genotypic differences were found in WUE values under terminal drought, *B.brizantha* CIAT 26110 cv. Toledo and *B. ruziziensis* 44-02 were the genotypes with lower values of WUE (9.85 g DM kg^{-1} H_2O and 10.63 g DM kg^{-1} H_2O, Figure 5) while *B. decumbens* CIAT 606 and Mulato CIAT 36061 showed higher values of WUE (13.65 g DM kg^{-1} H_2O and 12.8 g DM kg^{-1} H_2O, Figure 5). Although physiologically cv. Toledo presented low sensitivity to terminal drought, its final shoot biomass accumulation was lower than the genotypic mean. This is contrary to *B. decumbens* CIAT 606, with its ability to regulate stomatal behavior, transpiration rate and water use it could produce similar shoot biomass under the combined stress conditions compared with well watered conditions. This is because *B. decumbens* could match its growth rate with water availability while the hybrids cv. Mulato and Mulato II require more amounts of water to maintain their higher growth rates under drought stress (Figures 6 and 7). Relationship between the total dry matter (shoot + root) production and water consumed showed that cv. Mulato was outstanding in consuming the water and producing the shoot dry weight while. *B. ruziziensis* was least productive (Figure 6). Based on the values of relative reduction in shoot growth under drought stress compared with well watered condition, *B. decumbens* was better adapted to drought stress when combined with Al toxicity in soil (Figure 7). Mean daily transpiration rate was greater with cv. Mulato and *B. brizantha* cv. Marandu under well watered conditions while under drought stress the differences among the six genotpes were small (Figure 7).

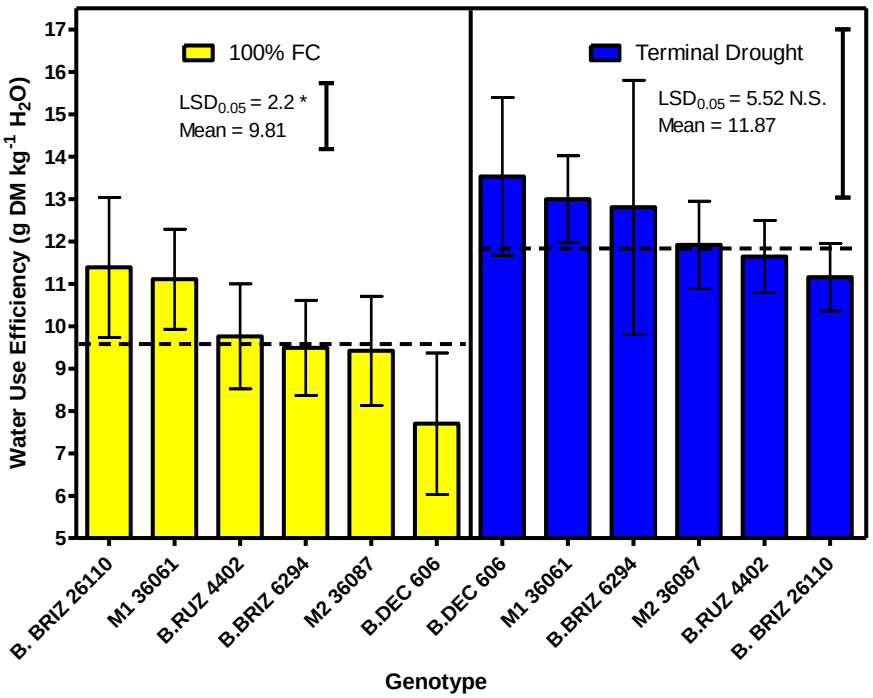


Figure 5. Differences in water use efficiency (unit of dry matter produced per unit of water used) among 6 *Brachiaria* genotypes subjected to 100% FC (well watered) and terminal drought stress to an Al-toxic soil.

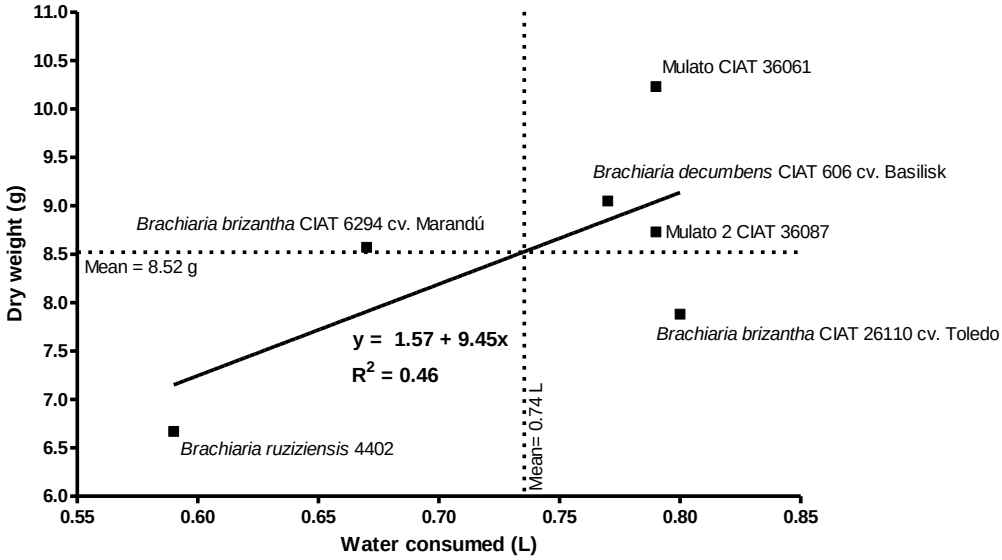


Figure 6. Relationship between total dry matter (shoot + root) production and water use of 6 *Brachiaria* genotypes subjected to combined stress conditions of terminal drought and Al toxicity. The genotypic mean for shoot dry weight = 7.89 and for water use = 0.621.

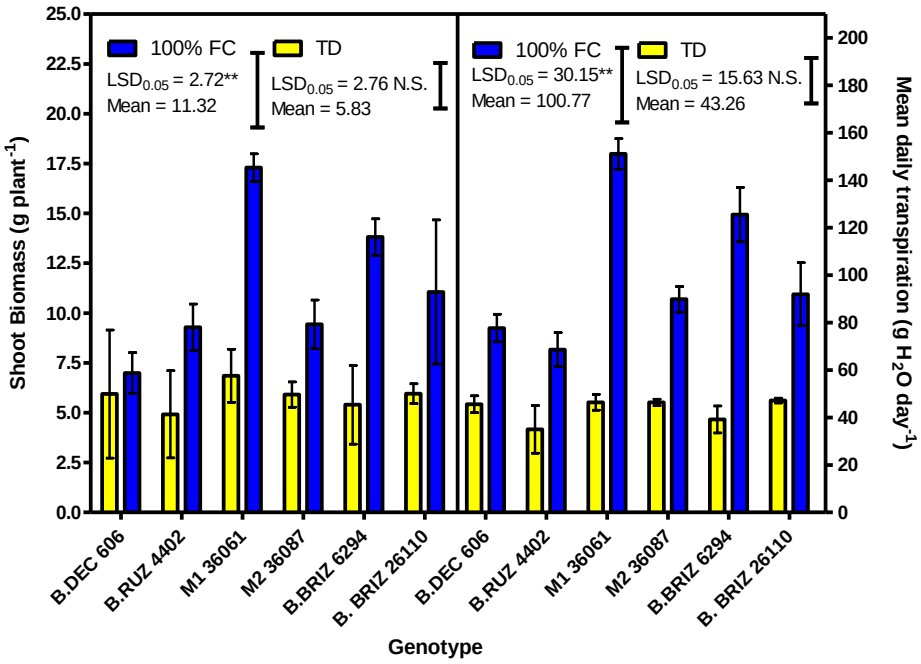


Figure 7. Differences in shoot dry matter and mean daily transpiration among 6 *Brachiaria* genotypes subjected to 100% FC (well watered) and terminal drought stress (TD) when grown in an Al-toxic soil.

Conclusions: Among the six *Brachiaria* genotypes that were subjected to a combined stress of drought and Al toxicity in soil, *B. decumbens* CIAT 606 and *B. brizantha* CIAT 26110 cv. Toledo were found to be superior in their ability to tolerate the combined stress conditions of terminal drought and Al toxicity. The superior performance of these two genotypes was attributed to a delay in stomatal closure combined with efficient use of the moisture stored in the soil during the dehydration process. Two genotypes, *B. brizantha* CIAT 6294 cv. Marandu and *B. ruziziensis* 44-02 were found to be sensitive to the combined stress conditions due to early stomatal closure that impacted their ability to use water to produce the shoot biomass. Two *Brachiaria* hybrids, cv. Mulato (CIAT 36061) and cv. Mulato II (CIAT 36087) showed greater demand for water with their higher growth rate and an intermediate type of response with moderate ability to adjust to the decreasing soil moisture.

5. Phenotypic differences in root development and distribution of eleven *Brachiaria* genotypes exposed to individual and combined stress of aluminum toxic acid soil and drought

Contributors: J. Polania, J. Miles and I. Rao

Rationale: Aluminum (Al) toxicity affects root development which in turn affects the acquisition of nutrients and water. There is very limited knowledge on the physiological and biochemical bases of *Brachiaria*grass' adaptation to either individual or combined stress factors of drought and Al toxicity. Seasonal drought affects both quantity and quality of forage in tropical savanna environments. *Brachiaria* grasses differ in their level of drought resistance. *B. brizantha* CIAT 6780 and *B. decumbens* CIAT 606 were found to be relatively more adapted to drought stress. Our objective was to determine phenotypic differences in root development and distribution of eleven *Brachiaria* genotypes that were exposed to individual and combined stress of aluminum toxic acid soil and drought stress conditions. This knowledge is needed to develop effective screening method(s) to evaluate *Brachiaria* hybrids generated by the *Brachiaria* breeding program at CIAT for their level of resistance to combined stress of drought and Al toxicity.

Materials and methods: A greenhouse study was conducted at CIAT-Palmira in 2009 using a soil from Matazul farm in the Llanos of Colombia. Plants were grown for 49 days in plastic cylinders (80 cm long with 7.5 cm diameter) that were inserted in PVC tubes. The trial included 11 *Brachiaria* genotypes: two apomictic Brachiaria parents (*Brachiaria decumbens* CIAT 606, *Brachiaria brizantha* cv. *Marandú* CIAT 6294), one sexual Brachiaria parent (*B. ruziziensis* 44-02), two commercial Brachiaria hybrids (*Brachiaria* hybrid cv. Mulato CIAT 36061, *Brachiaria* hybrid cv. Mulato II CIAT 36087), three apomictic hybrids from

BR02 population (BR02/1752, BR02/0465, BR02/1372), and three sexual hybrids from SX03 population (SX03/0846, SX03/0881 and SX03/2367) to determine genotypic differences in root development and distribution under individual and combined stress of Al-toxic acid soil and drought. The trial was planted as a randomized complete block arrangement with two levels of water supply: 100% field capacity (well-watered) and withholding of watering (to simulate terminal drought stress conditions) and two levels of fertilizer application to soil: high fertilizer application in which the soil was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo) and low fertilizer application in which the soil was fertilized with kg/ha of 20 P, 20 K, 47 Ca, 14 Mg and 10 S; as main plots and genotypes as sub-plots with three replications. Table 1 shows the details on soil characteristics. Treatments of water stress were imposed after 10 days of initial growth of plants that were established with stem cuttings. The initial soil moisture for all the treatments was of 100% field capacity. Plants with well-watered treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder. Plants with terminal drought were monitored for water stress by weighing each cylinder every two days for determination of decrease in soil moisture. Plants were harvested at 49 days after establishment, i.e., 39 days of withholding of water application.

A number of shoot traits were measured during the experiment, including total chlorophyll content (SPAD), photosynthetic efficiency, leaf conductance and rooting depth. At harvest time (49 days after planting; 39 days with water stress treatment), leaf area, shoot biomass distribution, and root traits were determined. The soil from the tube was removed and sliced into 6 layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75). Roots in each soil layer were washed free of soil and root length, mean root diameter, specific root length, and root dry weight, fine root proportion (root length with 0 and 0.5 mm of diameter/total root length x 100) were determined. Root length and mean root diameter were measured with an image analysis system (WinRHIZO, Regent Instruments INC). Root weight was determined after roots were dried in an oven at 60 ^{o}C for 48 h.

Table 1. Soil characteristics with high and low fertilizer application.

Fertilization level	pH	AL	Ca	Mg	K	P	Al saturation	Bulk density
		(cmol kg^{-1})				(mg kg^{-1})	(%)	(g cm^{-3})
Low	4.7	2.65	0.12	0.05	0.08	4.2	91	1.5
High	4.5	2.45	0.18	0.05	0.13	7.8	87	1.5

Results and discusión: During the plant growth and development the maximum and minimum air temperatures were 34 and 20 ^{o}C (Figure 1). The final soil moisture with terminal drought stress was at 56% of the field capacity. Significant genotypic differences were observed in leaf biomass under drought stress. Under irrigated and high fertilizer application conditions the genotypes CIAT 6294 and BR02/1752 were outstanding in their leaf biomass production, while under individual drought stress (drought with high fertilizer) the apomictic hybrids BR02/1372 and BR02/1752 were superior to other genotypes in leaf biomass production (Figure 2). The low fertilizer application significantly reduced the leaf biomass production. Under individual low fertilizer + irrigated conditions the genotypes SX03/2367, CIAT 36087 and BR02/1752 showed the highest leaf biomass. Under combined low fertilization and drought stress BR02/1752 and SX03/2367 were outstanding in leaf biomass production (Figure 2). The apomictic hybrid BR02/1752 was particularly outstanding in leaf production among genotypes tested under both fertilizer and water regimes.

Genotypic differences were observed in photosynthetic efficiency and leaf conductance at 44 days after planting under individual low fertilization and combined low fertilization and drought stress. Under individual drought stress conditions (high fertilizer + terminal drought) two genotypes BR02/1372 and BR02/1752 showed the lower values of leaf conductance than the others genotypes tested, indicating stomata control (Table 2). Under combined low fertility and drought stress conditions the genotypes that showed the regulation of stomatal conductance were SX03/0881, SX03/2367 and CIAT 6294.

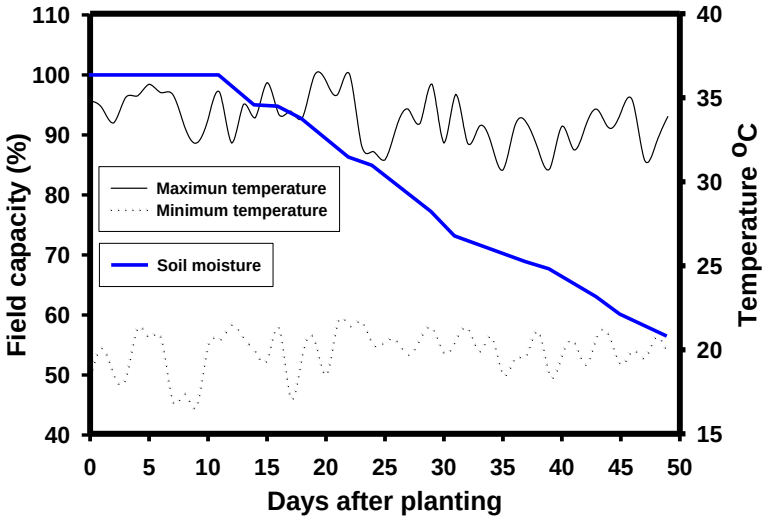


Figure 1. Soil moisture (field capacity), maximum and minimum temperature during soil drying and root development in soil tubes under greenhouse conditions of CIAT, Palmira.

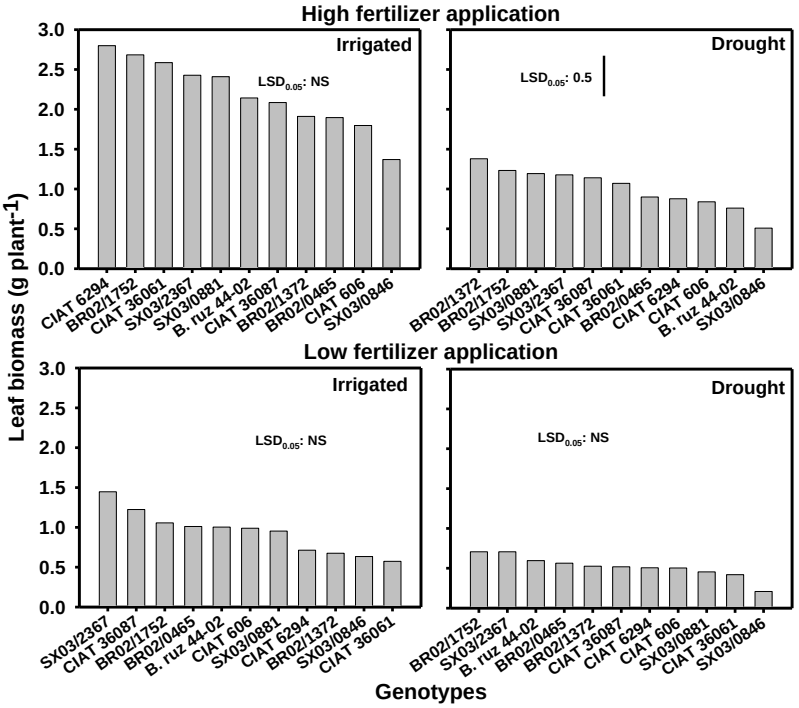


Figure 2. Influence of individual and combined stress of low soil fertility and drought on leaf biomass of 11 *Brachiaria* genotypes under greenhouse conditions of CIAT, Palmira.

Differences were observed in deep rooting among treatments. Under irrigated and high fertilizer application the genotypes CIAT 36061 and SX03/0881 showed greater ability for deep rooting at 43 days after planting than the other genotypes while the genotypes CIAT 606 and CIAT 6294 had lesser ability for deep rooting (Table 3). Three genotypes (CIAT 36061, SX03/0881 and BR02/1752) were outstanding in deep rooting ability under individual drought stress. Three genotypes (CIAT 36061, SX03/0881 and B. ruz 44-02) showed greater deep rooting ability than the other genotypes when grown under individual low soil fertility stress. The hybrids BR02/1752 and CIAT 36061 were outstanding in their deep rooting ability at 43 days after planting under combined low soil fertility and drought stress while SX03/0846, CIAT 6294 and CIAT 36087 showed lesser ability for deep rooting under combined stress conditions (Table 3).

Table 2. Influence of individual and combined stress factors of low soil fertility and drought on photosynthetic efficiency, leaf chlorophyll and leaf conductance of 11 *Brachiaria* genotypes that were grown under greenhouse conditions of CIAT, Palmira (I, irrigated and TD, terminal drought).

Genotype	Photosynthetic efficiency (fv'/fm')				Leaf chlorophyll content (SPAD)				Leaf conductance (mmol m^{-2} s^{-1})			
	High fertilizer		Low fertilizer		High fertilizer		Low fertilizer		High fertilizer		Low fertilizer	
	I	TD	I	TD	I	TD	I	TD	I	TD	I	TD
B. ruz 44-02	0.60	0.58	0.47	0.51	52.5	48.9	53.6	47.5	48.8	55.7	59.6	45.2
BR02/0465	0.56	0.58	0.59	0.59	50.6	45.2	43.6	46.6	76.4	35.7	58.7	54.5
BR02/1372	0.64	0.63	0.64	0.58	45.5	47.1	42.2	42.8	44.1	16.4	43.7	39.7
BR02/1752	0.58	0.54	0.59	0.59	46.4	49.8	43.5	46.8	28.7	16.2	39.0	36.4
CIAT 36061	0.56	0.56	0.58	0.54	51.0	54.4	49.6	54.6	62.9	34.7	47.1	59.8
CIAT 36087	0.49	0.55	0.63	0.54	48.3	46.9	46.5	45.9	65.6	46.4	36.3	34.9
CIAT 606	0.59	0.63	0.60	0.55	41.3	38.7	43.2	41.1	47.4	34.9	44.1	32.8
CIAT 6294	0.63	0.60	0.56	0.37	48.2	41.0	45.9	44.2	55.8	66.4	57.1	28.9
SX03/0846	0.61	0.61	0.63	0.57	43.0	44.2	46.5	41.0	71.4	43.2	39.6	35.4
SX03/0881	0.59	0.62	0.58	0.53	43.8	42.0	39.7	42.1	60.0	31.3	77.7	32.6
SX03/2367	0.62	0.58	0.61	0.53	52.6	47.5	47.0	52.9	58.6	24.5	67.7	30.2
Mean	**0.59**	**0.59**	**0.59**	**0.54**	**47.6**	**46.0**	**45.6**	**45.9**	**56.3**	**36.9**	**51.9**	**39.1**
$LSD_{0.05}$	**NS**	**NS**	**0.07**	**0.12**	**NS**	**NS**	**NS**	**5.5**	**NS**	**NS**	**28.9**	**25.4**

Table 3. Influence of individual and combined stress factors of low soil fertility and drought on deep rooting at 31 and 43 days after planting of 11 *Brachiaria* genotypes that were grown under greenhouse conditions of CIAT, Palmira (I, irrigated and TD, terminal drought).

Genotype	Deep rooting at 31 days after planting (cm)				Deep rooting at 43 days after planting (cm)			
	High fertilizer		Low fertilizer		High fertilizer		Low fertilizer	
	I	TD	I	TD	I	TD	I	TD
B. ruz 44-02	37	40	48	55	50	70	62	67
BR02/0465	35	35	41	31	41	67	44	53
BR02/1372	37	53	54	29	51	75	59	55
BR02/1752	**55**	**56**	**39**	**54**	**58**	**75**	**54**	**75**
CIAT 36061	55	56	61	52	66	75	75	74
CIAT 36087	32	38	29	25	48	70	53	38
CIAT 606	31	31	39	46	41	64	50	64
CIAT 6294	24	23	19	27	40	48	30	42
SX03/0846	33	32	31	32	47	50	38	48
SX03/0881	42	66	41	41	63	75	68	66
SX03/2367	31	61	34	39	52	74	60	63
Mean	**37**	**44**	**40**	**39**	**51**	**68**	**54**	**59**
$LSD_{0.05}$	**NS**	**23**	**NS**	**NS**	**NS**	**12**	**23**	**NS**

Significant genotypic differences were observed in total root length under individual terminal drought stress conditions and individual low fertility conditions. The apomictic hybrid BR02/1372 and the sexual hybrid SX03/2367 showed greater values of root length production under individual terminal drought stress conditions (Figure 3). The hybrids SX03/2367, BR02/1372 and BR02/1752 showed the best performance in root length development under low soil fertility conditions. Two apomictic hybrids (BR02/1752 and BR02/1372) and one sexual hybrid (SX03/2367) were found to be outstanding in their total root length production across soil depth under combined stress of low fertility and drought. The sexual hybrid, SX03/0846, showed the lowest value of total root length production among genotypes and

among soil fertility and water regimes tested (Figure 3). *B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294 were outstanding in thin root development with the highest values of fine root proportion than the other genotypes tested under control (high fertilizer + irrigated) and individual and combined stress factors of low fertility and drought (Figure 4).

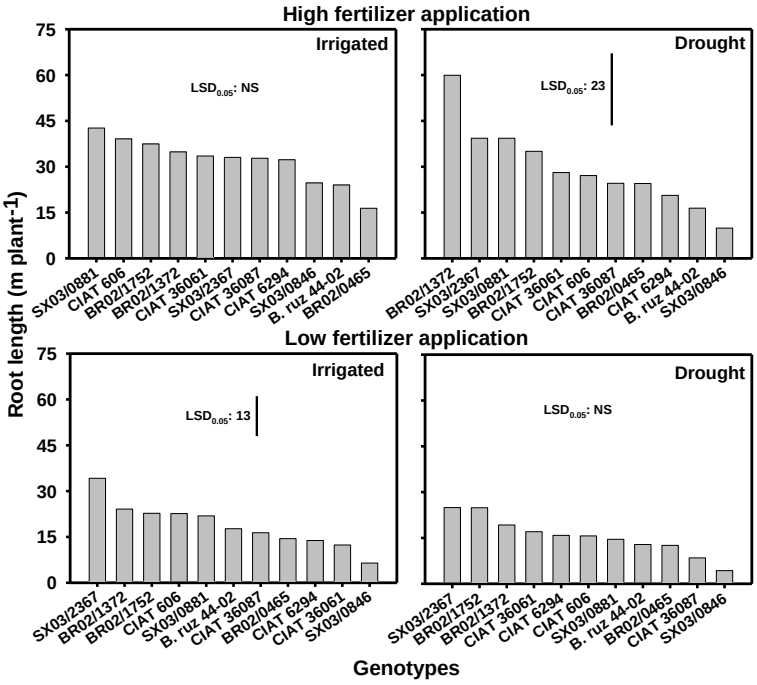


Figure 3. Influence of individual and combined stress of low soil fertility and drought on total root length of 11 *Brachiaria* genotypes that were grown under greenhouse conditions of CIAT, Palmira.

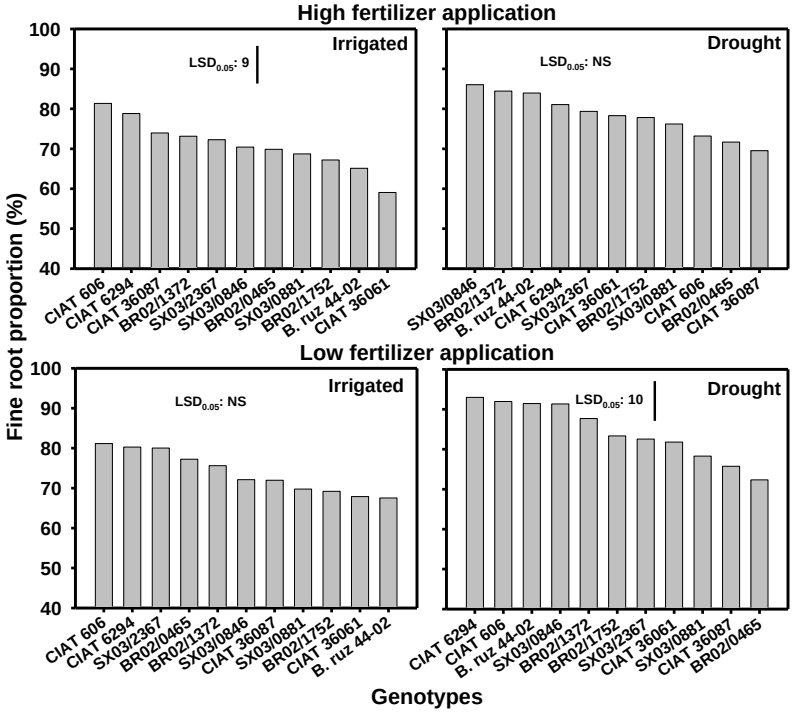


Figure 4. Influence of individual and combined stress of low soil fertility and drought on fine root proportion of 11 *Brachiaria* genotypes under greenhouse conditions at CIAT, Palmira.

Drought stress increased the root length development in deep soil layers than under irrigated conditions. The sexual hybrid, SX03/0881 and the apomictic hybrid, BR02/1372 (Figure 6) showed greater values of root length at soil depth of 60-75 cm under individual drought stress (Figure 5). The cv. Mulato (CIAT 36061) was the best in deep root development in terms of root length under low soil fertility conditions. The apomictic hybrid, BR02/1752 and cv. Mulato (CIAT 36061) were found to be outstanding in root length at soil depth of 60-75 cm under combined low soil fertility and drought stress indicating their superior adaptation to combined stress conditions (Figure 5). Two genotypes (CIAT 6294 and SX03/0846) showed poor development at soil depth of 60-75 cm than the other genotypes under individual and combined low soil fertility and drought stress conditions (Figure 5).

Conclusions: Results from this greenhouse study indicated that two apomictic hybrids (BR02/1752 and BR02/1372) and one sexual hybrid (SX03/2367) were outstanding in their total root length production across soil depth under combined stress factors of low soil fertility and drought.

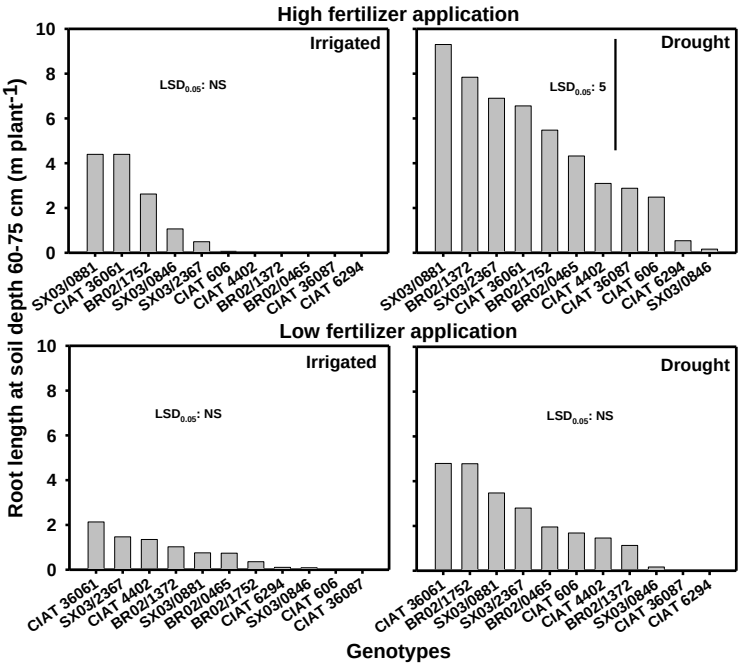


Figure 5. Influence of individual and combined stress factors of low soil fertility and drought on root length (at soil depth of 60-75 cm) of 11 *Brachiaria* genotypes grown under greenhouse conditions at CIAT, Palmira.

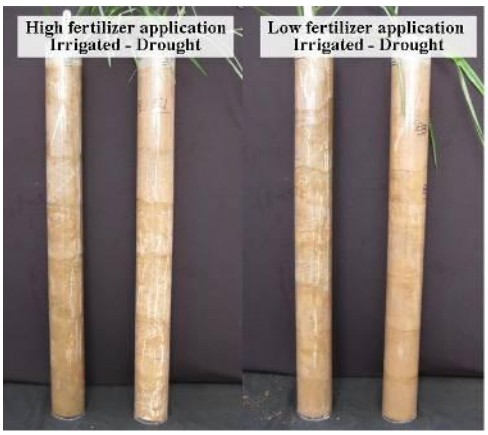


Figure 6. Influence of individual and combined stress factors of low soil fertility and drought on shoot and root development of an apomictic hybrid, BR02/1372, grown under greenhouse conditions of CIAT, Palmira (Photo by Jose Polania).

ANNEX 3

Project Title

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

Project Supported by
Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)

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FINAL REPORT

Project Title

Fighting drought and aluminum toxicity:
Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics

ANNEX 3
Functional genomics

ANNEX 3

Functional genomics

Output 3: *Genomic tools (gene libraries and cDNA microarrays) deployed to analyze the effects of drought and Al toxicity on expression of genes involved in root elongation, and to identify candidate genes responsible for drought and Al resistance in common bean, runner bean, and Brachiaria*

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1. Genomic tool development for Al and drought resistance in beans

1.1. Materials and Methods

Plant material and experimental conditions

Aluminum

Two bean genotypes were selected based on their contrasting response to aluminum (Al) toxicity. G 35346-3Q (*Phaseolus coccineus*), an Al-resistant genotype, and SER 16 (*Phaseolus vulgaris*), an Al sensitive genotype, were selected based on their root attributes (root length, number of root tips and vigor) in response to Al-toxicity under hydroponics conditions. The seeds were germinated for three days in a sandwich-system with sponge and paper sheets and using an acrylic frame as support. Seeds were then transferred to the nutrient solution containing 5 mM $CaCl_2$, 0.5 mM KCl, and 8 µM H_3BO_3 (Rangel *et al.,* 2005) with pH 4.5. Plants were adapted to the nutrient solution for 72 hours before the Al treatment which was added to a final concentration of 20 µM. The plants were grown in 18 L tanks on blocks with holes and supported with sponge. The experiment was established under greenhouse conditions and arranged in a randomized block design with four biological replications represented by four independent tanks. Root tips of 1 cm length were harvested at 0h (non-Al treated), 4h, 8h and 24h after the Al treatment. Root tips from each biological replication were put into independent 2 ml Eppendorf tubes and frozen immediately with liquid nitrogen prior to total RNA isolations.

Drought

Two bean genotypes were selected based on their contrasting response to drought stress. G 40159 (*Phaseolus acutifolius*), a drought-resistant genotype, and DOR 364 (*Phaseolus vulgaris*), a drought sensitive genotype, were selected based on their contrasting ability to develop a deep root system under drought conditions. The experiment was established under greenhouse conditions using plastic PVC-tubes filled with a specific soil:sand ratio and arranged in a randomized block design with three biological replications represented by three individual PVC-tubes. The genotypes were subjected to drought and irrigated (control) conditions. Irrigation was stopped at 10 days after seed germination to simulate natural drought stress. The control treatment was normally irrigated throughout the experiment. Two root portions, 5 cm top and 15 cm bottom, were harvested, washed with tap water and then frozen immediately with liquid nitrogen for the subsequent total RNA isolations. Harvests of root tissues were performed at 5 days intervals from 10 to 35 days of drought stress. An additional harvest at 6 days after germination was done as untreated control. Root tissues of the irrigated control were only collected at 20, 30, and 45 days after germination, which were representative to the stages of growing and flowering.

Total RNA and mRNA isolations: Frozen root tissues were ground mechanically to a fine powder using liquid nitrogen. Root tissues with their corresponding treatments and sampling times were processed separately. Total RNA isolations were carried out using the TRIzol® reagent (Invitrogen, Cat. # 15596-018) and following the manufacturer guidelines. Total RNA pellets were re-suspended in RNase-free water and quantified by spectrophotometer. Total RNA quality was determined through denaturing agarose gels (1.5%) containing formaldehyde and stained with ethidium bromide. For mRNA isolations of the Al experiment 60 µg of total RNA from each genotype and sampling time for the control (non-Al treated) and Al treatments were pooled within each target genotype. For the drought experiment 10-20 µg of total RNA from each genotype, root portion and sampling time for the drought and irrigated treatments were pooled. mRNA isolations were performed from 240 µg of high-quality total RNA for the Al experiment and 150-300 µg for the drought experiment using the MicroPoly(A)Purist™ Kit (Ambion, Cat.# AM1919) and eluted in 200

µl of RNA storage solution. The eluted mRNA was quantified with the NANODROP 1000 Spectrophotometer (Thermo Scientifc) and then concentrated through ethanol precipitation to get a final concentration of 500 ng/µl.

cDNA synthesis: Single-stranded (ss) and double-stranded (ds) cDNA were synthesized with the SMART™ PCR cDNA Synthesis Kit (Clontech, Cat.# 634902). An initial amount of 0.5 µg of mRNA was used in the synthesis of sscDNA. sscDNA amplification was performed by Long Distance (LD) PCR following the kit user manual. LD PCR was optimized at 17 PCR cycles obtaining cDNA smears around 0.2-10 kb.

cDNA subtraction and PCR-enrichment of differentially expressed cDNA: cDNA subtraction was carried out with the PCR-Select™ cDNA Subtraction Kit (Clontech, Cat.# 637401) following the user manual guidelines. Both forward and reverse subtractions for the Al experiment were performed using the G 35346-3Q genotype as tester and the SER 16 genotype as driver in the forward subtraction, and the SER 16 genotype as tester and the G 35346-3Q genotype as driver in the reverse subtraction. For the drought experiment forward and reverse subtractions were also performed using the G 40159 genotype as tester and the DOR 364 genotype as driver in the forward subtraction, and the DOR 364 genotype as tester and the G 40159 genotype as driver in the reverse subtraction. The PCR enrichment of the differentially expressed sequences was done with two rounds of amplifications with 27 and 12 PCR cycles. The subtraction efficiency test was done with the housekeeping genes actin and β-tubulin. The secondary PCR products enriched for differentially expressed sequences were cloned into the pGEM-T Easy Vector (Promega, Cat.# A1360) and then transformed into *E. coli* DH5α electrocompetents cells.

Colony PCR to determine length of inserts and polymorphism of the subtracted libraries: PCR of 20 and 10 randomly selected transformants for Al and drought respectively were performed using the pGEM-T Easy T7 and SP6 universal primers. The trasnformants were sequenced and then analyzed to establish the redundancy of the subtracted library.

*Differential screening:*The screening of the differentially expressed genes were done following the protocols of the PCR-Select Differential Screening Kit (Clontech, Cat.# 637403) and the DIG DNA labeling Kit (Roche, Cat.# 11175033910). cDNA hybridization probes for the Al experiment consisted of 1) forward subtracted cDNA (G 35346-3Q as tester), 2) forward unsubtracted cDNA, 3) reverse subtracted cDNA (SER 16 as tester), and 4) reverse unsubtracted cDNA. For the drought experiment hybridization probes were the following: 1) forward subtracted cDNA (G 40159 as tester), 2) forward unsubtracted cDNA, 3) reverse subtracted cDNA (DOR 34 as tester), and 4) reverse unsubtracted cDNA. Exposure to X-way films were carried out for 1 minute, 5, minutes, 15 minutes and 30 minutes.

1.2. Results and Discussion

More than 1,000 clones from bean and *Brachiaria* treated with both drought stress and Al toxicity were identified from unique genetic resources that were never used in this type of work. These genomic tools including the differentially expressed genes using the gene pools will be used to create high dense maps for bean and *Brachiaria* and to identify major QTL for the key traits. Using gene pools we conducted differentially expressed gene analysis to identify candidate genes for Al resistance in bean and *Brachiaria* (shown in Section 3. Genetic tool development and isolation of Al resistance genes in *Brachiaria*).

Three differentially expressed genes were found from the Al- induced subtractive cDNA library (Table 1) and 1 gene was found from the drought-induced subtractive cDNA library (Table 2) in common bean. C15 and C2 were previously reported as stress inducible genes associated with the abiotic stress tolerance. Further confirmation of gene expression under Al toxicity is underway.

In order to find whether the candidate genes isolated from this subtraction screening and 4 *MATE* and 2 *STOP1* homologous genes, which are mentioned in the section: 2. Target gene approach to identify Al resistance genes in common bean, are related to QTL (quantitative trait loci) for Al-resistance), we have predicted the location of the candidate genes on *P.vulgaris* chromosome using the synteny between soybean and common bean (Geleano et al. 2009). The predicted location of the candidate genes were matched to the location of QTL for Al-resistance, which is reported by Yan et al. (2004), Liao et al. (2004), Beebe et al. (2006) and Lopez-Martin et al. (2009) (Table 3).

Table 1. List of differentially expressed genes up-regulated by Al toxicity in the Al-resistant bean genotype G 35346-3Q (*P. coccineus*) ND: Not determined in this study

Clone ID (identified length)	BLAST search annotation	GO molecular function	Reported as a gene related to
C12 (553bp)	Similar to DEX1 (Deffective in exine formation 1)	calcium ion binding	ND
C15 (524bp)	Similar to SCPL (serine carboxypeptidase-like)	serine carboxypeptidases	Biotic and oxidative stress response in rice (Liu et al, 2008)
C20 (396bp)	Similar to U3 small nucleolar RNA-associated protein 11	U3 small nucleolar RNA-associated protein 11, Utp11	ND

Table 2. List of differentially expressed genes up-regulated by drought stress in the drought-resistant bean genotype G 40159 (*P. acutifolius*)

Clone ID (identified length)	BLAST search annotation	GO molecular function	Reported as a gene related to
C2 (269bp)	Similar to ATMBF1C/MBF1C (Multiprotein bridging factor 1C)	transcription coactivator/ transcription factor	Thermotolerance in Arabidopsis (Suzuki et al, 2008) Tolerance to environmental stress in Arabidopsis (Suzuki et al, 2005)

Table3. Predicted location of the candidate genes related to Al-tolerance in *P. vulgaris* chromosome.

Query id	hit chromosome in Soybean	% identity	E value	bit score	predicted position in *P.vulgaris* chromosome (Geleano et al. 2009)	possible QTL name
C12	Gm01	91.32	3.00E-167	592	10	-
	Gm03	90.91	3.00E-162	575		
C15	Gm12 Gm11		1.10E-49	201.5	6,11	-
C20	Gm05	90.91	2.00E-24	117	2.4.6	Pup2.1 (Beebe et al. 2006)
	Gm08	89.29	3.00E-21	106		
	Gm16	87.06	7.00E-18	95.3		
C2	Gm06 Gm12				11	-
MATE-a	Gm13	85.92	2.00E-34	150	1, 5, 6, 9, 11	-
	Gm15	86.23	3.00E-33	147		
	Gm09	86.13	3.00E-33	147		
	Gm04	82.73	7.00E-25	119		
MATE-b	Gm13	90.24	7.00E-54	215	5, 7	Nrt5.1 (Lopez-Martin et al. 2009) Tae5.1 (Yan et al. 2004)
	Gm13	98.96	4.00E-41	172		
	Gm12	89.7	3.00E-52	209		
	Gm12	97.92	2.00E-39	167		
	Gm12	98.04	4.00E-16	89.8		
	Gm10	88.41	7.00E-49	198		

Table3. Predicted location of the candidate genes related to Al-tolerance in *P. vulgaris* chromosome.

Query id	hit chromosome in Soybean	% identity	E value	bit score	predicted position in *P.vulgaris* chromosome (Geleano et al. 2009)	possible QTL name
MATE-c	Gm15	93.37	2.00E-63	246	6, 11	-
	Gm13	86.11	2.00E-44	183		
	Gm13	93	2.00E-33	147		
MATE-d	Gm10	95.59	5.00E-55	219	unknown	-
	Gm10	92.09	3.00E-47	193		
	Gm02	95.59	5.00E-55	219		
PvSTOP1-1	Gm16	89.17	0	1232	7	-
	Gm20	82.31	0	832		
	Gm10	81.82	0	804		
PvSTOP1-2	Gm20	92.77	0	1254	7	-
	Gm10	92.81	0	1168		

1.3. Publications

Blair M.W. and M. Ishitani. 2009. Common bean: a model food legume for international agriculture.
Grain Legumes 53: 8-9

2. Targeted gene approach to identify Al resistance genes in common bean

2.1. Materials and Methods

Sequence homology search and phylogenetic analysis: 164,500 EST sequences of *P.vulgaris* were collected from GeneBank (www.ncbi.nlm.nih.gov). Homologous sequences for citrate transporter gene and *STOP1* gene were searched by tBLASTn against the EST sequences, using with the following peptide sequences as queries, *HvAACT1* gene (Accession number; BAF75822) and *STOP1* gene (Accession number; NP_001031140, NP_849746.1 and NP_174697.1). Amino acid sequences of hit genes in BLAST search were aligned by PRANK program (http://www.ebi.ac.uk/goldman-srv/prank/prank/) and the phylogenetic tree was constructed using the NJ method to find candidate homologous sequences in common bean.

Plant materials, growth condition and RNA isolation: Common bean (*Phaseolus vulgaris*) genotypes proved to be Al-sensitive (genotype VAX-1) and Runner bean (*Phaseolus coccineus*) genotypes proved to be Al-tolerant (genotype G35346-3Q) were chosen according to the published results by Rangel *et al.,* 2005. They were also described as P-efficient and P-inefficient cultivars respectively under low P condition (Yan *et al.,* 2004). The seeds were germinated on Canadian peat in pH 5.5 and incubated for 3 days. Subsequently, they were transferred to the nutrient solution containing 5mM CaCl, 0.5mM KCl, and 8 µM H_3BO_3 (*Rangel et al.,* 2005). During 3-days culture, the pH in the solution was gradually adjusted from pH 5.5 to pH 4.5 with HCl. Afterwards, Al was added into the nutrient solution to a final concentration of 20µM at pH 4.5. It was confirmed that the pH (4.5) in the solution after Al addition kept stand during the culture.

Root tips (10 mm length from the bottom) were harvested at 0h (non-Al treated) and 4, 8 and 24 hours after the Al treatment. Root tips were frozen in liquid nitrogen before grinding and then stored then at -80°C for the subsequent total RNA isolations. RNA isolations were performed with a Trizol-based protocol following the manufacturer guidelines.

More than three hundred mg of fresh weight root tips were obtained from plants for one eppendorf tube. Root tips were harvested from the four biological replications. Root samples were frozen immediately after the harvest.

Quantitative real-time PCR: Total RNA was isolated from the root tips as described above. The isolated RNA was treated with DNase I and then first strand cDNA was synthesised using with the SuperScript III Reverse Transcriptase (Invitrogen, www.invitrogen.com). Random hexamer primers were used for this purpose. The reaction was stopped by heating at 70°C for 15 min. Quantitative

real-time PCR (qRT-PCR) was undertaken using the Stratagene MX-3000p (www.stratagene.com). The SYBR Green detection system was used with Brilliant® II SYBR® Green QPCR Master Mix (Stratagene, www.stratagene.com). The constituents of the qRT-PCR reaction mix were 1x Master Mix, 150 nM each forward and reverse primers, 1 ul of synthesized cDNA template and Ultra pure DNAse/RNAse-free distilled water in a final volume of 20 µl. The qRT-PCR cycling stages consist of initial denaturation at 95°C (10 min), followed by 40 cycles of 95°C (30 sec), 57-62°C (60 sec) depend on the target genes, 72°C (60 sec), and a final melting curve stage of 82°C (1 sec), 55°C (30 sec) and 95°C (30 sec). The fluorescence signal was recorded during the strand elongation step at 72°C and the melting curve stag. Samples for qRT-PCR were run in three biological replicates and two technical replicates. Relative gene expression was calculated using the comparative $\Delta\Delta C_T$ method according to Livak and Schmittgen (2001). Control plants of the Al-sensitive genotype VAX-1 were used as calibrator and the actin gene was used as internal standard. The PCR efficiencies of the actin and the target genes were comparable and thus relative gene expression was calculated without efficiency correction. The primers for expression analysis by qRT-PCR were designed by vector NTI software (invitrogen) according to the nucleotide sequences encoding the putative *MATE-b*, *MATE-c* and *PvSTOP1-1* genes.

2.2. Results and Discussion

Targeted gene approach allowed us to identify genes associated with physiological and molecular mechanisms such as release of organic acids from roots in response to Al toxicity in beans and *Brachiaria*. Some of the key genes including transporter and regulatory components such as transcription factor were identified as described in Figure 1. These genes will further contribute to understanding the resistance mechanism in the crops through molecular and genetic studies.

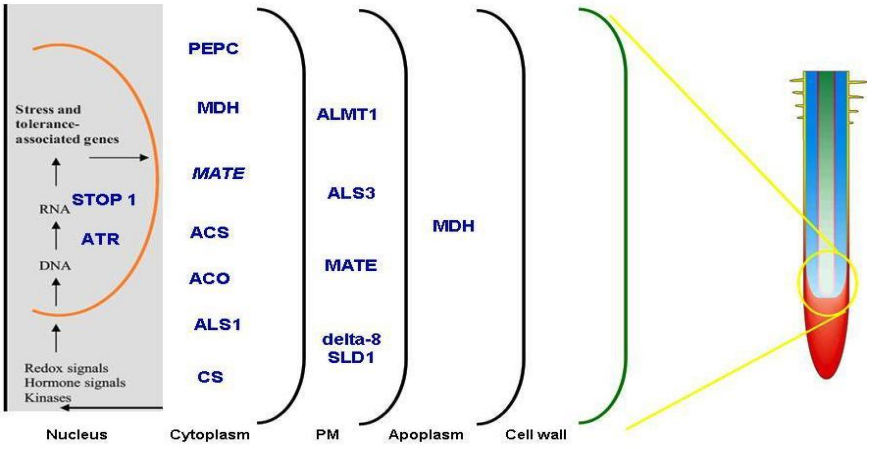

Figure 1. Key genes known to be involved in Al-resistance in plants and their respective putative sub-cellular localizations

Aluminum (Al) resistance in common bean is known to be due to exudation of citrate from the root after a lag phase. The aims of this study were to identify the genes, which have the important role for exudation of citrate and to compare its functions between an Al-resistant (G35346-3Q, *Phaseolus coccineus)* and an Al-sensitive (VAX-1, *Phaseolus vulgaris*) genotype. The citrate transporter gene MATE is a member of a large gene family that is known to regulate exudation of citrate in other plants. One of C2H2-type zinc finger transcription factors (*STOP1*) was identified as an upstream regulatory factor for *AtALMT1* and plays a critical role in Arabidopsis conferring tolerance to major stress factors in acid soils. In this experiment, we identified putative *MATE* genes related to the citrate transporter and putative *STOP1* genes in common bean through BLAST search. Several ESTs of *P. vulgaris* which have similarity with known *MATE* genes were gathered and aligned to assess their homology. Based on the alignment result four homologous sequences were identified (*MATE-a*, *MATE-b*, *MATE-c* and *MATE-d*). *MATE-a, MATE-b, MATE-c* and *MATE-d* have the predicted amino acid sequence similarities of 55%, 61%, 68% and 64%, respectively, with the *HvAACT1* gene, which is one of the aluminium inducible-citrate transporters. *MATE-a* and

MATE-b have the predicted amino acid sequence similarities of 66% and 73%, respectively, with the Arabidopsis *FRD3* (ferric reductase defective 3) gene (Locus: AT3G08040), which is one of iron inducible-citrate transporters. Furthermore, the phylogenetic tree has shown that sequences of *MATE-a* and *MATE-b* are in the category of the iron inducible-citrate transporters (FRD3) and *MATE-c* and *MATE-d* sequences are in the clade of the aluminium inducible-citrate transporters (Figure 2). Two *STOP1* homologous sequences were also identified as *PvSTOP1-1* and *PvSTOP1-2* from ESTs of *P. vulgaris*. *PvSTOP1-1* and *PvSTOP1-2* have the predicted amino acid sequence similarities of 61% and 68%, respectively, with the Arabidopsis *STOP1* gene.

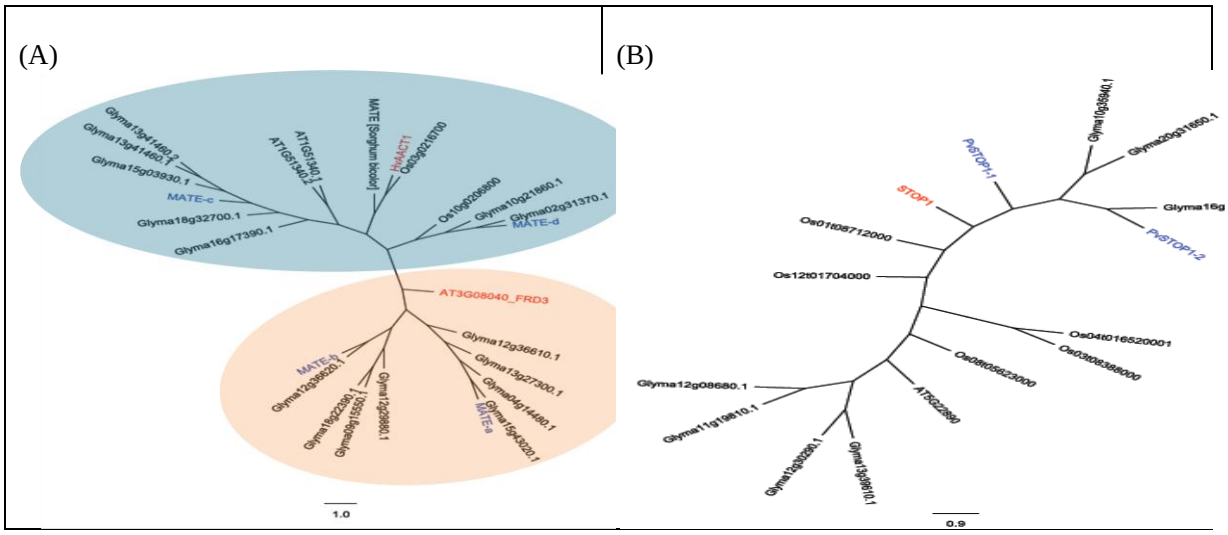

Figure 2 . Phylogenetic tree of *MATE* genes (A) and *STOP1* genes (B). The predicted amino acid sequences of the *MATE* genes and *STOP1* genes were aligned by PRANK program and the phylogenetic trees were constructed using the NJ method. Genes in blue circle or in red circle are similar to aluminium inducible-citrate transporter (*HvAACT1*) or iron inducible-citrate transporter (*FRD3*), respectively.

Gene expression analysis using qRT-PCR was carried out for both *MATE-b* related to the iron inducible-citrate transporter and *MATE-d* related to the aluminium inducible-citrate transporter. The expressions for both genes were greatly enhanced by Al treatment for 4 hours in both bean genotypes However, upon extended Al treatment duration, the expression of *MATE-b* and *MATE-d* continued to increase in the Al-sensitive genotype VAX-1, but not in the Al-resistant genotype G35346-3Q (Figure 3A and 3B). The expression of PvSTOP1-1 which might be an upstream regulatory factor involved in Al tolerance was also determined (Figure 3C). Early Al stress (4h) induced the expression of *PvSTOP1-1* gene in both bean genotypes. But, while with Al treatment duration, it decreased the expression level in G35346-3Q while it continued to keep the higher expression level in VAX-1.Rangel et al. (2009) clearly demonstrated that Al treatment induced the exudation of citrate in both Al-resistant and Al-sensitive bean genotypes after 4h. However, while the resistant genotype consequently recovered from Al stress by 24h, the sensitive genotype recovered transiently. Those results show that *MATE* genes and *STOP1* genes in common bean are clearly responded to Al toxicity and in common bean also *MATE* genes might be responsible for Al-induced citrate exudation.

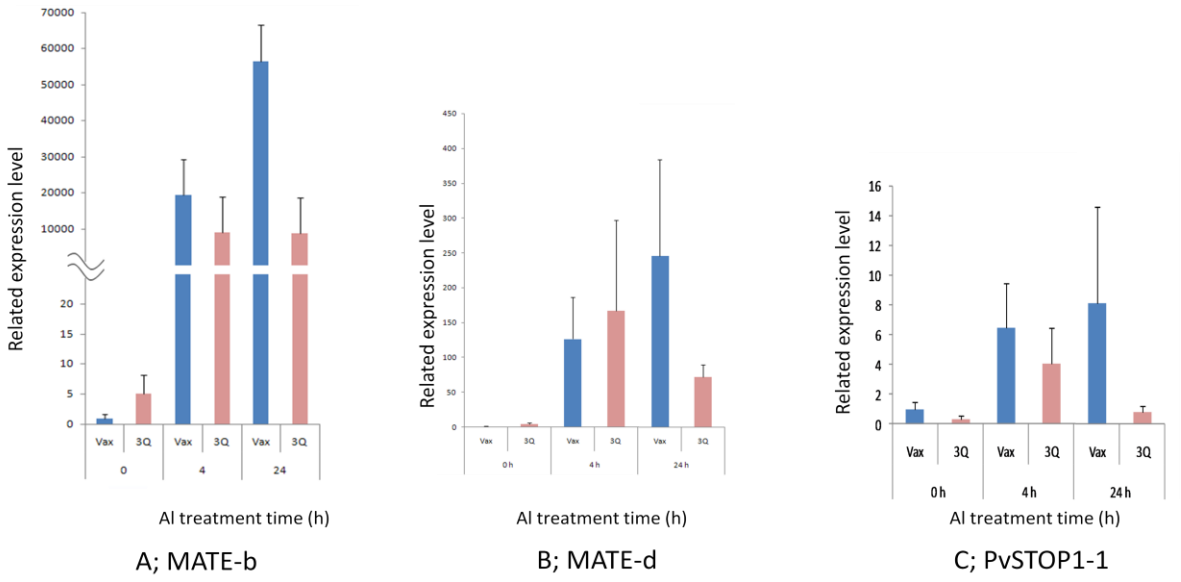

Figure 3. Expression of two *MATE* genes (A; *MATE-b*, B; *MATE-d*) and a *STOP1* homologue gene, (C; *PvSTOP1-1*) under extended duration of Al treatment in the bean genotypes G35346-3Q (3Q; *Phaseolus coccineus*, Al resistance) and VAX-1 (VAX; *Phaseolus vulgaris*, Al-sensitive) grown in nutrient solution treated without or with 20 µM Al for up to 24h. Total RNA was extracted from root tips. Quantitative RT-PCR was performed using the actin gene as internal standard and untreated plants of VAX-1 as calibrator. Relative gene expression was calculated from three biological and two technical replicates.

1.1.3. Publications:

One article is under preparation for publication related to the data above.

3. Genetic tool development and isolation of Al resistance genes in *Brachiaria*

3.1 Materials and Methods

Plant Material: Seeds of *B. decumbens* and *B. ruziziensis* were germinated in 200 µM $CaCl_2$ (pH 4.2) in the greenhouse for 4-5 days. Similar seedlings with root length between 4 and 5 cm were subjected to Al treatment with continuously aerated solutions consisting of 200 µM $CaCl_2$ (pH4.2) with and without 200 µM $AlCl_3$. Root tips (1 cm length) of *B. decumbens* and *B. ruziziensis* were collected at 0, 3, 6, 24 and 72 hours after treatment.

Candidate genes for Al resistance: A set of 18 candidate genes previously identified from a cDNA subtractive library between the resistance genotype *B. decumbens* and the sensitive genotype *B. ruziziensis* were selected and characterized in this study.

Total RNA isolations and cDNA synthesis: Total RNA was isolated from root tips using Trizol® (Invitrogen, USA) following the manufacturer's protocol. Total RNA was treated with DNase I (Invitrogen, USA) to remove genomic DNA. cDNA for PCR experiments was synthesized using the SuperScript III reverse transcriptase (Invitrogen, USA). We used a co-amplification reverse transcription (Co-RT) strategy for priming cDNA. This technique combines oligo-(dT) with 18S-RNA-specific primer in the initial reverse transcription reaction.

RACE (Rapid Amplification of cDNA Ends): This technique uses the strategy of PCR for the amplification and quick isolation of specific cDNAs or genomic DNA. This technique avoids the construction and screening of genomic DNA libraries, which sometimes is very complicated and consumes lots of time and resources. The protocol to obtain the cDNA fragments was the one provided with the BD SMART TM RACE cDNA Amplification kit (Catalog #634914, BD Biosciences Clontech) and the 5' RACE 2.0 kit (Invitrogen).

Plasmid preparation and transformation into E. coli: The fragments from the purified cDNA-RACE were sub-cloned into the pGEM-T Easy vector (Promega) taking advantage of the adenine residual *Taq* polimerase leaves at the 3' ends. The ligation reaction was done according to specific instructions given by the kit's manufacturer. The electroporation method was used for transformation of plasmid DNA into *E. coli* cells. Selection of positive clones (plasmids that contain

the specific insert) was done through the induction of the β-Galactosidase gene in plates containing LB / Amp / IPTG / X-Gal.

Gene expression analysis: Comparative expression analysis of seven differentially expressed genes were carried out in *B. decumbens, B. brizantha* cv Marandu, *Brachiaria* Hybrid Mulato II and *B. ruziziensis* at 0, 3, 6, 24, 48 and 72 hours of Al-exposure. cDNA from root tips was evaluated by Rea-Time PCR using gene-specific primers. We also assessed the performance of these genes in leaves of seedlings under Al-stress conditions at 0, 6, 24 and 48 hours of exposure. Real-Time PCR was carried out in the MJ research Opticon II as follow: 20 µl reaction volume containing 10 µl Master Mix (2X) SYBR Green I Kit (Stratagene, USA), 175 nm of each primer, and 5 µl of 1:10 diluted cDNA template. PCR cycling conditions were 95^{o}C for 10 minutes, followed by 40 cycles of 95^{o}C for 10 seconds, 50-60^{o}C for 20 seconds and 72^{o}C for 30 seconds. The fluorescence reading was done at 72^{o}C and 83^{o}C and specificity of amplified products were confirmed by a melting curve from 65^{o}C to 95^{o}C.

Data analysis: Sequence analysis and homologous searches were performed with Vector NTI (Invitrogen, USA) and the BLAST algorithm of NCBI. We used the qBase software v 1.3.5 (http://medgen.ugent.be/qbase) to analyze Real-Time PCR data. The software employs a delta-Ct relative quantification model with PCR efficiency correction and multiple reference gene normalization. To estimate efficiency of PCR amplification of each gene, a standard curve was prepared using a serial dilution of cDNA or plasmid DNA carrying the target gene as insert. We used 18S-rRNA gene for normalization.

3.2. Results and Discussion

3.2.1. Genomics tool development and differently expressed genes under Al stress

The 7 differentially expressed genes found in *B. decumbens* -AlBdec3, AlBdec5, AlBdec8, AlBdec10, AlBdec14, AlBdec15, and AlBdec16 were evaluated in four *Brachiaria* materials at different times of Al exposure (0, 3, 6, 24, 48 and 72 hours). In general terms, similar results were observed in the expression of these genes in the three most tolerant species (*B. decumbens, B. brizantha* cv Marandu, and *Brachiaria* Hybrid Mulato II) (Figure 1).

We got several contigs by RACE, which allowed us to deduce the complete sequence of the AlBdec10 gene (Figure 2). The BLAST algorithm enabled us to confirm that this sequence really corresponds to the Metal-dependent protein hydrolase family. A probable function for the HD domain of this family corresponds to a signal transduction. This family of proteins contains a large number of metal binding residues. Within this family are the phosphohydrolases, which usually are metalloenzymes that contains distinctive combinations of metal-chelating residues, typically histidines and aspartates.

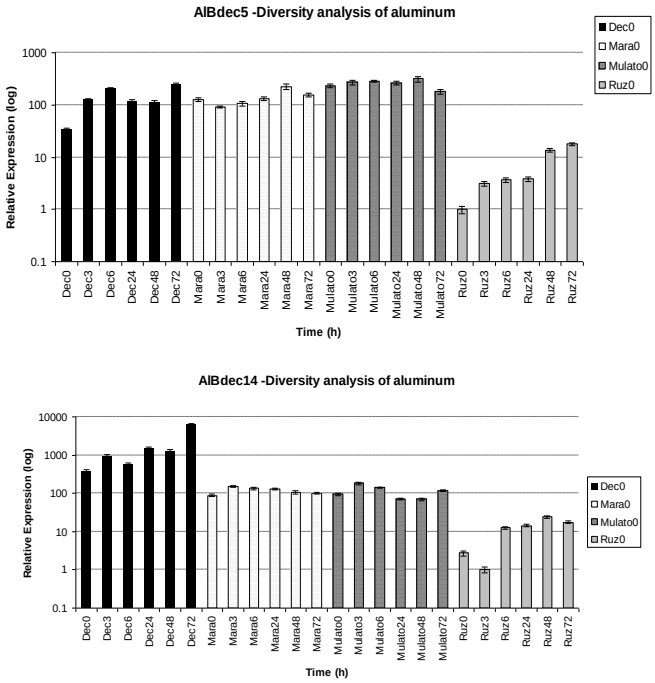

Figure 1. Gene relative differential expression obtained by Real-Time PCR from *Brachiaria* root tips. Black bars, *B. decumbes*; white bars, ***B. Brizantha*** cv Marandu; gray bars with white points, *Brachiaria* Hybrid Mulato II, and gray bars, *B*. *ruziziensis*. Logarithmic scales are presented (0, 3, 6, 24, 48 and 72 hours respectively).

TGTCACAGCGAGCCGTCACGTCTCCTCTCCTTGGCATCGGCGGGCGATCCCAAATCCGGAACCCTTTCCCCACCATGG
CGTCTCTCTCGCCGGCAGCCGCCTCCTCGCCCAAGAGGCTGCGGGTCTACTCGTCCGCCCCCACCCCCACCGACGGC
GACGGGAGCGGCAAGCGCGTGGGGACCCACAASGGCAGCTTCCACTGCGACGAGGCGCTCGGCTGCTTYCTYATCCG
CCTCACCTCCCARTTCRCCGGCGCYGACGTCRTCCGCACCCGCGACTCSCAGATCCTTGATACACTGGATGCCGTGCT
TGATGTTGGTGGTGTCTATGATCCCAGCCGGCACCGCTATGATCATCATCAGAATGGCTTCAGTGAGGTTTTTGGACAT
GGATTCAACACAAAACTTAGCAGTGCTGGACTTGTGTACAAGCATTTTGGTAAGGAGATAATTGCTAAGGAGCTTGGGG
TTAATGAGGACCATGAAGATGTTCACCGCTTGTACCTTGCAATATATAAAAGCTTTGTTGAGGCACTTGACGCGATTGAT
AATGGAATCAATCAATACGACACAGACCAACCGCCAAAGTATGTGAACAATACACACTTGTCKYSRSGTGTTGGGCGCC
TTAATCCGGACTGGACTGATCCAGACCAGTCACCTGAGAAGGAGAATGCAGCATTTCAACAGGCAATGATGCTTGCTGG
AAGTGAATTTATGGAGAGTGTTCGCTTTCATGTTAAATCATGGTTACCTGCAAGATCTATTGTCCTGGAGTGTTTGCTATC
AAGAGGAAAGGTTGACCCAAGTGAAGAAATCATGGTTTTGGATAGATTCTGCCCGTGGAAGCTTCATCTATTTGAGCTTG
AAGAGGAGCTGAAGATTGATCCTCTGACCAAGTATGTGCTTTATCAGGATGAGAGGAGCMAGAGCTGGCGAGTGCAAG
CYGTTGSTGTTGCTCCYGACAGGTTCGAGAGCCGAAAGGCTCTGCCAGAGAAGTGGAGGGGCATGAGAGACGATGAAC
TGTCTGCAGAAACTGGCATTCCCGGCTGTGTGTTTGTCCATATGAGCGGTTTCATTGGGGGCAACAAGACCTACGAGGG
AGCGTTGGAAATGGCGAGAGCTGCTCTGAAATGCTGATTGAACCAAGGCACTTCCAGTAACAGTCTTTCCCCATGTTAC
CATTGGTTTAGTAAACACATCAGAGTTTCAGCAAACNCAGTCTGAAATTGGGCCTTCNCCCATGTTATCTTTGACCCATC
TTCGTAGCTAACATTGAGAATACTCATTCTGTTATCTTCGTCTTACAGAAGAGGTCTTAATTAAAATTTTCCGCCTTTGTAT
TATCTGGTAATGAGCTAGAAGGTGACAATTTATTTTAAAAAAAGTGTTGGAACATATTAAAAAAAAAAAAAAAAAAAAAA
AAAA

Figure 2. Alignment of three contigs of the *AlBdec10* gene showing punctual variations in some nucleotides. Polymorphisms are highlighted in yellow. The start codon is highlighted in blue, while the stop codon in fuchsia.

3.2.2 Target gene approach in Brachiaria

a) STOP1 homologs in Brachiaria

Recently, a C2H2-type zinc finger transcription factor (*STOP1*) was identified as an upstream regulatory factor for AtALMT1. Iuchi *et al.,* 2007 evaluated the expression level of *STOP1* at various pH and Al treatments in *Arabidoposis* (Col-0 (WT)) by quantitative RT-PCR. Although their results indicated that *STOP1* plays a critical role in *Arabidopsis* conferring tolerance to major stress factors in acid soils, they could not concluded that STOP1 was involved in the mechanism of the phenotypic variations of Al tolerance in *Arabidopsis.*

We found a partial sequence homologue of STOP1 gene from cDNA of *B. decumbens*. We designed a stock of specific PCR primers obtained from consensus sequences of this gene reported for different species in the gene bank (www.ncbi.nlm.nih.gov). By doing a Blastx and Tblastx analysis with the sequenced PCR products discovered as a query, we identified similarity with *STOP1* gene from Rice and *A. thaliana* (Figure 3).

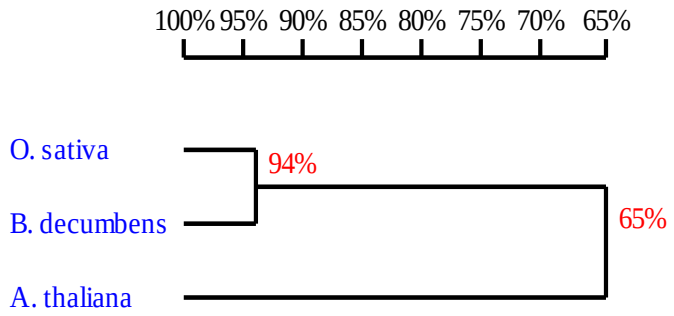

Figure 3. Similarity dendogram between translated PCR products sequences from *B. decumbens* and STOP1 protein sequences from *O. sativa* and *A. thaliana*.

2) Aluminum-Activated Citrate Transporter in Brachiaria

We identified in *B. decumbens* a PCR product homologue to the HvAACT1 gene (Figure 4), which is responsible for the Al-activated citrate secretion in barley (*Hordeum vulgare)*. This gene belongs to the multidrug and toxic compound extrusion (MATE) family and was constitutively expressed mainly in the roots of an Al-resistant barley cultivar (Furukawa *et al.,* 2007) and in apices of rice bean (*Vigna umbellata*) roots (Yang *et al.,* 2006). This agrees with the data obtained by Wenzl *et al.,* (2002) who assure that *Brachiaria* species might employ citrate and others organic acids to bind and detoxify aluminium within root apices.

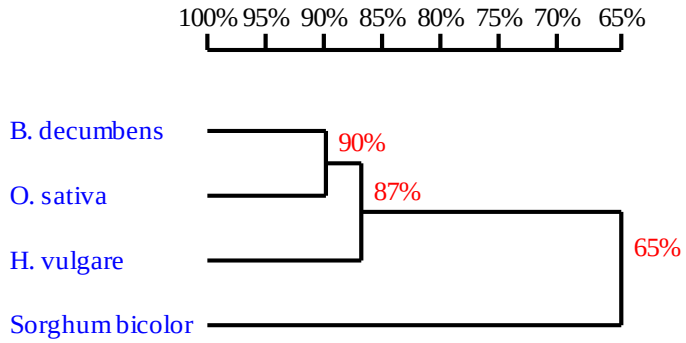

Figure 4. Similarity dendogram between translated PCR products sequences from *B. decumbens* and MATE protein sequences from *O. sativa, H. vulgare* and *A. thaliana*.

3) ALMT homologs in Brachiaria

The release of organic anions from roots has been implicated as a mechanism to protect plants from aluminum (Al) toxicity. We are examining whether homologues of TaALMT1 wheat gene are present in *B. decumbens* and whether they have differential expression between *B. decumbens* (tolerant) and *B. ruzisiensis* (susceptible). We were able to isolate two partial TaALMT1 homologues (Figure 5).

```
T. aestivum TVVVVMEYTVGATLSKGLNRALATLVAGCIAVGAHQLAELAERCGDQGEPIMLTVLVFFV  148
Secale cerea TVVVVMEFTVGATLSKGLNRALATLVAGCIAVGAHQLAELTERCSDQGEPVMLTVLVFFV  141
B. decumbens MQYIYAQLFAGATLSKGLNRALATLVASSLAIGAHELASLIVPTSEQAEFILLVVFVFFV   94
B. decumbens TVVVVMEFTVGATLSKGLNRALATLVASSLAIGAHELASLIVPTSEQAEFILLVVFVFFV   60
H. vulgare  TVVVVMEYTVGCTLSKGLNRAFATLVAGFIAVGAHQVAN...RCGAQGEPILLAIFVFFL  157

T. aestivum ASAATFLRFIPEIKAKYDYGVTIFILTFGLVAVSSYRVEELIQLAHQRFYTIAVGVFICL  208
Secale cerea ASAATFLRFIPEIKAKYDYGVTIFILTFGLVAVSSYRVEELIQLAHQRFYTIVVGVFICL  201
B. decumbens ASAATFSRFIPEIKARFDYGVSIFILTFSLVAVSSYRVEELMPLALQRISTIFVGVAICL  154
B. decumbens ASAATFSRFIPEIKARFDYGVSIFILTFSLVAVSSYRVEELMPLALQRITTIFVGVAICL  120
H. vulgare  ASAATFSRFIPEIKARYDYGVTIFILTFSLVAVSSYRVEELIQLAHQRFSTIVIGVLTCL  217

T. aestivum CTTVF  213
Secale cerea CTTVF  206
B. decumbens CTTVF  159
B. decumbens CTTVF  125
H. vulgare  CTTIF  222
```

Figure 5. Comparison of the deduced amino acid sequences of candidate genes found at the GenBank.

3.2.3. Root elongation rate under different stress condition and gene candidate expression

We also evaluated the impact of different doses of aluminum on the development or growth of the roots of Brachiaria in hydroponic systems (data not shown). Likewise, we evaluated the effects of other types of stress over root development to understand stress specific response in two contrasting Al resistant genotypes. Stress caused by 200mM NaCl inhibited completely the growth of the roots in both species, while 100mM NaCl resulted in a 50% inhibition of *B. decumbens* and only 35% in *B*. *ruziziensis*. This trial also showed that the pH did not have a significant effect on root development of *B. decumbens* and *B*. *ruziziensis*. At least, under our experimental conditions we did not obtain differences in growth when seedlings were exposed to pH 4.2 or pH 6.0 (data not shown).

The results showed that the expression of genes in leaves is not consistent with the expression in the tips of the roots. In some cases, there was no differential expression between *B*. *decumbens* with respect to *B. ruzisiensis* (Figure 6). This was also observed in the expression of these genes in root tips of seedlings under stress by NaCl (Figure 6).

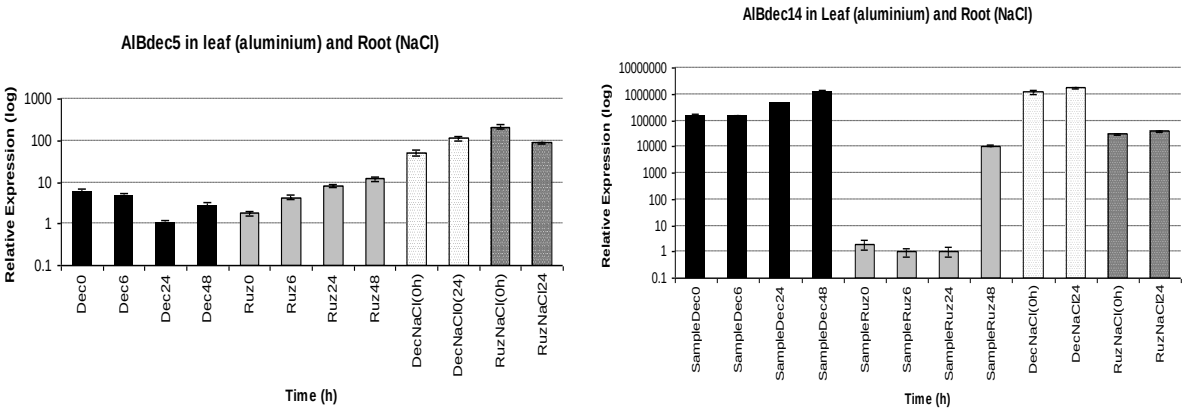

Figure 6. Gene relative differential expression obtained by Real-Time PCR from leaves (seedlings in Al solution, Black bars= *B. decumbens* and gray bars= *B*. *ruziziensis*) and root tips (seedlings in NaCl solution, white bars= *B. decumbens* and gray bars with white points = *B*. *ruziziensis*).

3.3. Publications

One article is under preparation for publication related to the data above.

4. Identification and mapping of QTLs associated to Aluminum resistance in *Brachiaria ruziziensis* x *Brachiaria decumbens* hybrid population

Contributors: Harold Suárez-Baron, Gerardo Gallego, Sergio Mejia, Maryam Chaib, Felipe Gutierrez, Jaime Vargas, Jaumer Ricaurte, Myriam Duque, John Miles, Idupulapati Rao and Joe Tohme

Abstract: At acid pH, the phytotoxic Al^{3+} cation is released into soil solution, where it inhibits root growth, hindering the ability of plants to acquire water and nutrients. The grasses belonging to the *Poaceae* family, especially those tetraploid species that belong to *Brachiaria* genus, exhibit strong resistance to the extremely restrictive conditions that characterize acid soils. Given that, in the present study, the aluminum resistant response was evaluated with quantitative trait loci (QTLs) analysis. First an interspecific highly heterozygous *Brachiaria decumbens* (resistant) x *Brachiaria ruziziensis* (susceptible) F1 population was generated. Then, it was phisiologically and phenotipically characterized after an exposition period to highly concentrated aluminum hydroponic solutions. Then the hybrid population was genotyped by means of SSR, EST, SCAR and AFLP markers. Single dose markers (SDM) were used for linkage map construction with 122 markers. This map was used for QTL analysis that correlated phenotypic data with molecular information, obtaining five putative QTLs that explained the 6.6%, 7.8%, 11%, 13% and 21% of the aluminum stress response respectively

Key Words: Al resistance, QTL mapping, Abiotic stress, Brachiaria.

Abbreviations: Al – Aluminum; ***Bd* –** *Brachiaria decumbens;* ***Br* –** *Brachiaria ruziziensis ;* **QTL -** Quantitative trait loci ; **LG –** Linkage group; **EST -** Expressed sequence tag; **AFLP -** Amplified fragment length polymorphism; **CAPS -** Sequence characterized amplified region; **SSR -** Single sequence repeat; **IM –** Interval mapping; **CIM –** Composite interval mapping; **RL -** Root length; **RD -** Root diameter; **cM –** Centimorgan; **LOD score -** Logarithmic odds score; **CIAT –** International Center for Tropical Agriculture

Introduction

The Aluminum (Al) is the most abundant metal and third most abundant element in the earth´s crust; plants have evolved in a soil environment where the roots are potencially exposed to high concentrations of aluminum. Fortunately, phytotoxic forms of Al are relatively insoluble at alkaline, neutral, or mildly acidic soil pH values. However, at soil pH 5.0 or lower, toxic forms of Al are solubilized and accumulate to concentrations that inhibit root growth and function (Buchanan *et al,* 2000).

Assessing this characteristic is of vital importance due to the fact that acid soils make up about 40% of world´s arable land (3.95 billions of ha), and almost 40% of this lies in the American continent, including almost the whole Amazon basin (Arango 2004). These types of soils are distinctive of a main portion of tropical and subtropical regions. In Colombia, for instance, 57% of the agricultural land is acidic (Cochrane 1991), and most of the plants cultivated in this soils present symptoms like gradual reduction in nutrient acquisition, inhibition of root development and drought susceptibility. Also, other symptoms are observed like severe damage to the ultrastructure of peripheral root cap cells, cells of epidermis and of peripheral cortical layers within the meristematic and elongation root zones (Ikeda and Tadano 1993; Ciamporova *et al*, 2000).

For these reasons, it is important to study species with adaptation to acid soils with high concentrations of aluminum and their ability to grow normally under nutrient-poor condition. Grasses belonging to the *Poaceae* family, especially those tetraploid species that belong to *Brachiaria* genus, exhibit a strong resistance to the extremely restrictive conditions that characterize acid soils. *Brachiaria* is an important source of forage grasses used in cattle raising in tropical Latin America. This genus contains several commercial species, including *B. decumbens, B. dictyoneura, B. humidicola, B. brizantha, and B. ruziziensis,* each of which has been released in one or more tropical American countries. For the Aluminum resistance, the species *Brachiaria decumbens* (2n=2x=36) is well-adapted, not like *B. ruziziensis* (2n=2x=18) that does not have Aluminum tolerance.

In our particular case, the research is devoted to the identification of molecular markers and QTLs related to genes of agronomic importance in the genus *Brachiaria;* in this study with a special emphasis in Aluminum resistance. At the present, the species within *Brachiaria* are the most widely used as forages in the world, especially in Central and South America; in Brazil, Colombia and Venezuela there are approximately between 30 and 70 million hectares cultivated with these

species (Miles *et al*. 1998; Pizarro *et al.* 1998), from which 85% belong to *Brachiaria decumbens* cv. Basilik y *B.brizantha* cv. Marandú (Keller-Grein *et al.* 1998). In South Pacific and Asia, these species occupy nearly 300.000 ha in the humid and sub-humid tropics (Rao *et al.* 1996; Sturr *et al.* 1998).

The research objectives were: (1) to build a linkage map for Brachiaria and identify molecular markers strongly associated with resistance to aluminum and (2) to identify QTLs for Al tolerance in *Brachiaria ruziziensis* x *Brachiaria decumbens* hybrid population.

Materials and Methods

Plant Material

A subset of 176 F_1 hybrid lines from a cross between *Brachiaria decumbens* cv. Basilisk (accession: CIAT/606) resistant to Al toxicity and *Brachiaria ruziziensis* (accession: CIAT/44-02) susceptible to Al toxicity were used in the present study. For the development of this population it was used as a male parental *Brachiaria decumbens* (CIAT/606), this is a genotype with apomictic reproduction and a female parent *Brachiaria ruziziensis* (CIAT/44-02), accession that is characterized by sexual reproduction and has become a tetraploid accesion by Swenne *et al.* (1981) using an induction treatment with colchicine. The population was developed by the breeding Program of Tropical Forages at CIAT.

Screening for Al resistance

The parental lines and progenies were screened for Al resistance in the laboratory of drought and Phytonutrition (CIAT) by Mejia (2007), using a nutrient solution with all nutrients (Table 1), the two treatments used were 200 μM of aluminum ($AlCl_3$) + 200 μM of $CaCl_2$ and the other without the presence of Al (non-stress or control conditions). The nutrient solution was changed daily in order to avoid fluctuations in nutrients and pH of the solutions was adjusted daily to 4.2 with 1 N NaOH or 1 N HCl. The experiment was arranged as a randomized design with 2-6 replications. The harvest of the plants was carried out at 3 weeks after undergoing the genotypes to treatments and 6 cycles were performed for evaluation. Two phenotypic variables were evaluated for Al resistance: root length (RL) and mean root diameter (RD). The ratio of average root length under stressed versus control conditions for each repetition was used as measure of Al resistance.

Table 1. Concentration of nutrients in hydroponic solution, used in the rooting phase (9 days)

Nutrient	Concentration
NH_4NO_3	500 μM
KNO_3	300 μM
$Ca(NO_3)_2$	200 μM
NaH_2PO	5 μM
$MgCl_2$	90 μM
$MgSO_4$	60 μM
$FeCl_3$	5 μM
Na_2EDTA	5 μM
H_3BO_3	6 μM
$MnSO_4$	1 μM
$ZnSO_4$	1 μM
$CuSO_4$	0.2 μM
Na_2MoO_4	1 μM
Na_2SiO_3	5 μM
NaCl	55 μM
***HCl**	1N

*Concentration of HCl to adjust the pH to 4.2

Genotypic analysis

Total DNA was extracted from fresh leaf tissue using approximately 0.03g. The pulverized plant material was transferred to a microtube and 600 ul of preheated buffer-CTAB (65 ^{o}C) solution was added. The tube was vortexed for a few seconds and incubated at 65 ^{o}C for 10 min. Following incubation, 600 ul of chloroform/isoamyl alcohol (24:1) was added and the mixture was shaken vigorously. The extract was centrifuged for 10 minutes at about 12,000 r.p.m. and the supernatant transferred to a new microtube. The chloroform/isoamyl extraction step was repeated twice and 1 ml of 100% cold ethanol was added to the final supernatant. A DNA pellet became visible upon gentle swirling. It was transferred into a new tube, rinsed two times with 70% ethanol and dissolved in 100 ul of 10 mM TE buffer solution containing 10mg/ml of RNAse.

A total of 345 markers, including ESTs (selected from a subtractive library), AFLP and SSR,were tested in the parents CIAT/606 and CIAT/4402, in order to standardize the amplification conditions, and select the methodology for marker evaluation in the hybrid population. Those markers that did not show polymorphism in the PCR amplification were evaluated as CAPS markers.

Data Analysis and Segregation Analysis

For each one of the markers, polymorphic bands were selected between the susceptible parent (44-02) and the tolerant one (606). Taking these bands into account it was determined its presence (1) or absence (0) in the segregating population.

In order to make the segregation analysis, it was employed the methodology proposed by Wu *et al* (1992), used for simple dose markers (SDM) in the generation of linkage maps of polyploid species. The markers present in a parental with a segregating proportion 1:1 were included in the analysis; for this discrimination it was used a hypothesis test of X^2 with a significance level of 0.05. The markers that presented proportions significantly different to the expected were skipped from the linkage analysis.

Linkage map and QTL analysis

To the original matrix data with the information generated by the markers with segregation 1:1 (a total of 122 markers), it was added it´s inverse matrix (mirror). To calculate the distances and the organization of the markers it was employed the program MapMaker/EXP v 3.0b for Macintosh (Lander *et al.* 1987). Map distances were calculated in centiMorgans (cM) and using the Kosambi mapping function (Kosambi 1944), in order to correct the distance of the map, calculated in the recombination percentage.

QTL analysis was performed using the phenotypic information from root length (RL) and mean root diameter (RD) and according with the methods of Interval Mapping (IM) and Composite Interval Mapping (CIM), using WinQTLCartographer v.2.5 for windows (Wang *et al.* 2005). Based on a chromosome number of 18 and the observed map length of 1094 cM, a LOD score of 2.5 was selected as the threshold for declaring presence of a QTL to reduce the experimental false-positive rate to $P<0.05$ (Lander and Bostein 1989) and 1000 permutations.

Results and Discussion

Phenotypic evaluation for tolerance to Al resistance

There were highly significant differences between genotypes (Figure 1) in the two variables. There were hybrids found to exhibit length and diameter variation with values higher or lower than those displayed by the parental species, which indicated high variability and the presence of transgressive segregation in population, according with the findings by Buitrago (2003) and Arango (2004) evaluating a population derived from the same cross in nutrient solutions.

The root length is strongly affected by the high concentration of Al in the solution, obtaining an average of 5.7 m plant-1 without Al and 2.7 m plant-1 in presence of Al. The diameter increased, going from 0.30 without Al to 0.39 mm plant-1 with Al.

The mean, range and distribution for the trait of root length (RL) for the hybrid population and their parents are summarized in Figure 2. Analysis of variance (ANOVA) showed the presence of significant genetic differences between the two parents and among hybrid progenies for this specific trait.

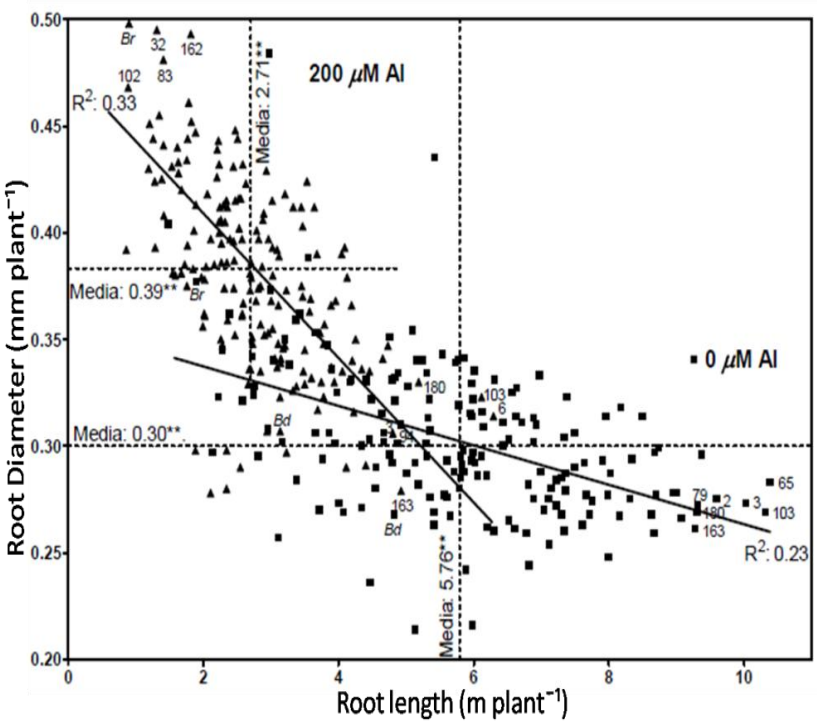

Figure 1. Root length and diameter in *Bd, Br* and the hybrid population. Plants were evaluated in a nutritive solution with Al and other without Al (used as control), for a 3 weeks period.

B. decumbens showed a higher root length (RL) than *B. ruziziensis*, thus confirming the resistance of *B. decumbens* to aluminum toxicity. The frequency distribution of root diameter (RD) of hybrid progeny did not fit a normal distribution (data not shown). However, the broad range of phenotypic variation suggests that this trait is quantitative in nature.

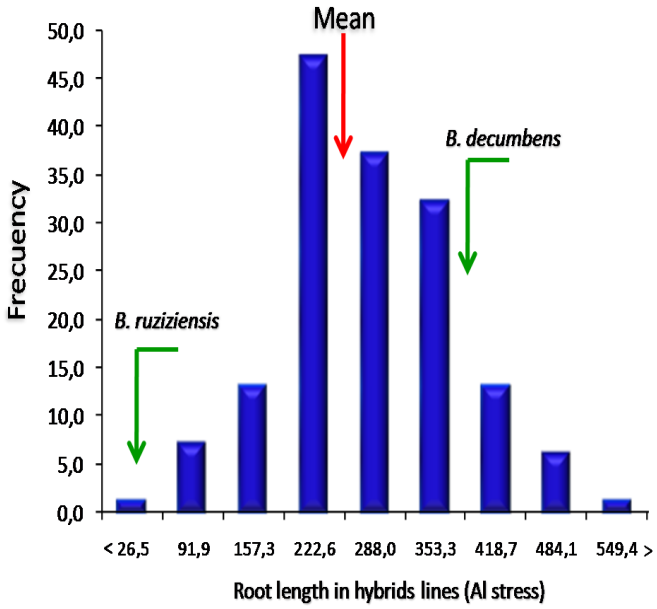

Figure 2. Phenotypic distribution for root length trait of hybrid progeny derived from the cross of *B. decumbens* x *B. ruziziensis*; Mean: average value of 175 hybrid lines.

Marker segregation and genetic linkage map construction

Three hundred and eighteen markers showing clear polymorphisms between the two parents were chosen for the progeny survey. The segregation of the 122 markers that were included in the genetic linkage map was tested for goodness of fit using the X^2 test with the 1:1 ratio expected, respectively, for marker loci that were either polymorphic or shared between parents. Subsequently, these loci were mapped and used for QTL analysis in the hybrid lines (Table 2). On average, the non parental and null alleles (complete absence of signal) were detected among the progeny at a frequency of 5.3 %. These cases were treated as missing data. Map distances, expressed in centiMorgans (cM), were calculated by the Kosambi´s mapping function (Kosambi 1994). Eighteen linkage groups were obtained covering 1094 cM (mean, 60.7 Cm and 6.7 markers per group) (Figure 3).

Table 2. Summary of the molecular linkage map information. For each linkage group shows the number of mapped markers on each system and the total distance given in centimorgans.

			Marker System			
Linkage group	**cM**	**Number of Markers**	**SSR**	**AFLP**	**EST**	**SCAR**
1	161.0	17	3	10	4	0
2	34.0	4	2	2	0	0
3	97.0	11	1	9	1	0
4	88.0	11	0	10	0	1
5	16.0	3	1	2	0	0
6	34.0	5	1	4	0	0
7	43.0	7	1	5	1	0
8	15.0	4	1	3	0	0
9	65.0	8	2	4	2	0
10	94.0	8	3	5	0	0
11	40.0	4	2	2	0	0
12	76.0	5	4	1	0	0
13	51.0	4	3	1	0	0
14	47.0	4	0	3	1	0
15	29.0	5	0	2	3	0
16	89.0	8	0	8	0	0
17	53.0	8	0	8	0	0
18	62.0	6	0	6	0	0
Total	**1094**	**122**	**24**	**85**	**12**	**1**

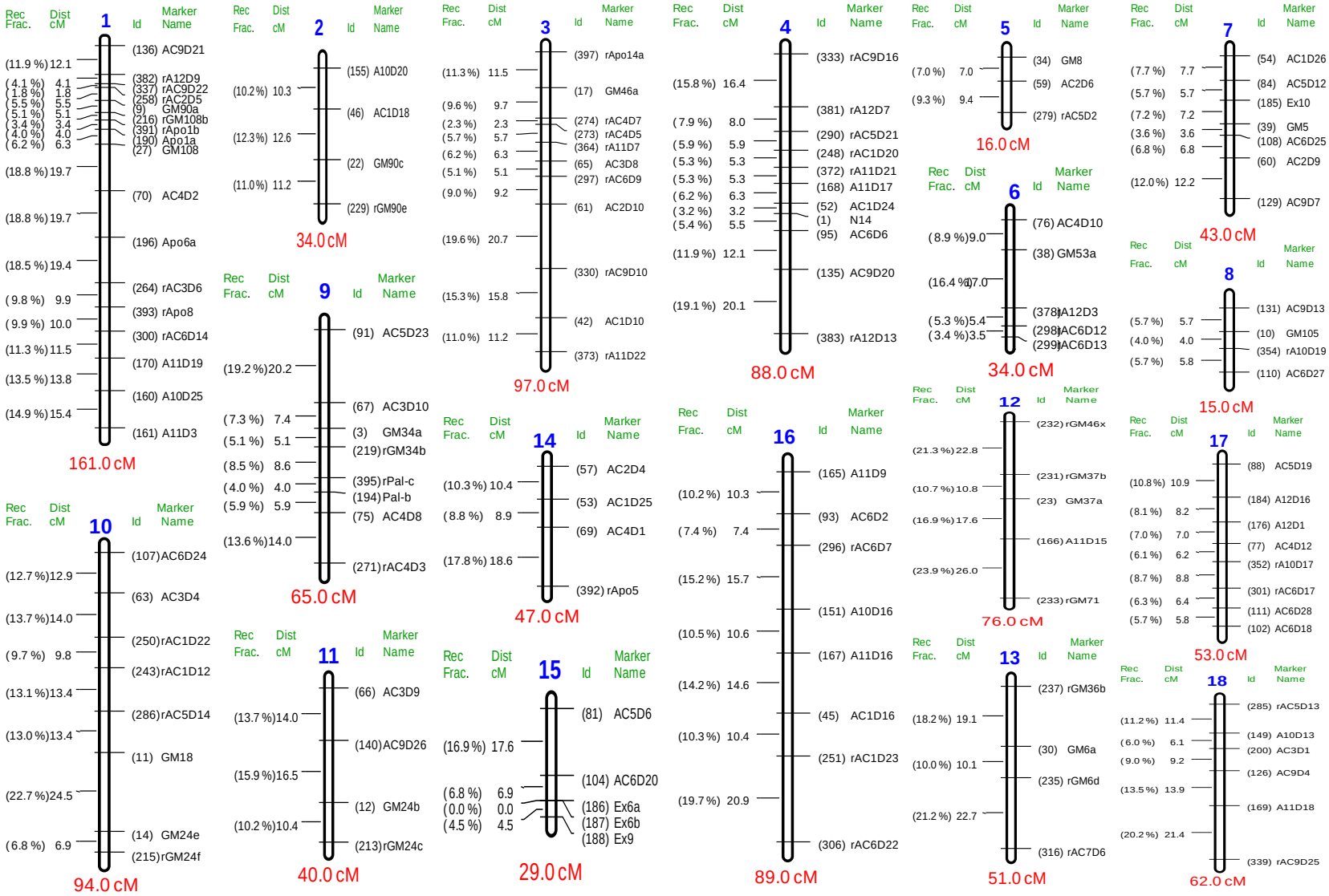

Figure 3. The molecular linkage map with 18 linkage groups and 122 marker loci constructed from 175 hybrid lines derived from the cross of *Bd* x *Br.* Linkage group numbers are indicated at the top. The distance between markers is given in cM. Numbers below each group indicate the length of the linkage group in cM.

QTL mapping for Al resistance

The genetic map obtained was constructed with the purpose of making an approach to elucidate the molecular basis of aluminum stress resistance in *Brachiaria decumbens*, a species considered the most resistant to this abiotic factor within the genus. For QTL analysis it was employed genetic and phenotypic information of 175 genotypes, using the statistic association between molecular markers and phenotypic variables (root length and root diameter).

Transgressive selection was observed for root length, this property was established for the presence of individuals from the progeny with phenotypes more extreme than those observed in both parents (*Bd* and *Br*).

Through the analysis of interval mapping (with statistical significance of α = 0.05), were determined a total of five QTLs related to root length (Figure 4 and 6). Using the method of composite interval mapping (using a significance of α = 0.05), was confirmed the association between this trait and those five putative QTLs (Figure 5 and 6).

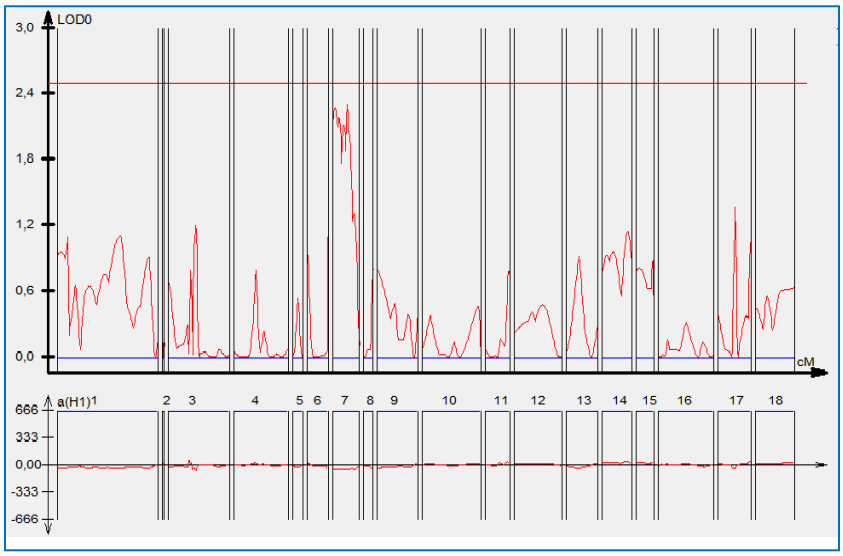

Figure 4. Graphical representation of interval mapping analysis (IM), showing the results for all linkage groups included in the QTL evaluation. Peaks are statistically significant when exceeding the critical LOD value established (α = 0.05) and using 1000 permutations. Red line indicates the root length trait.

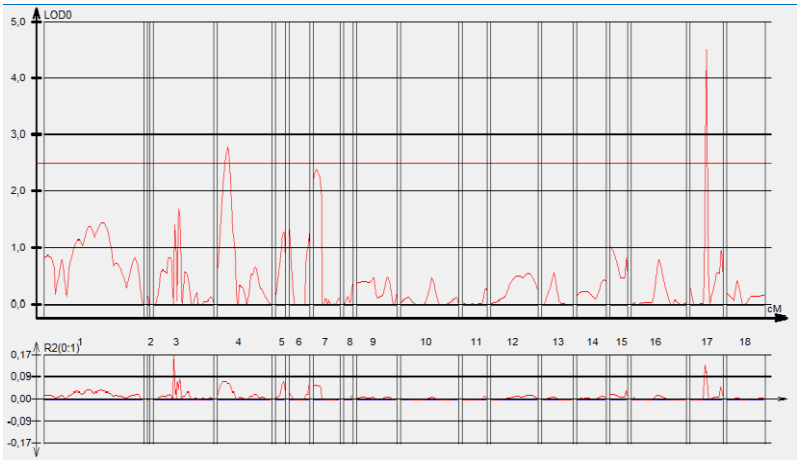

Figure 5. Graphical representation of interval composite mapping analysis (CIM), showing the results for all linkage groups included in the QTL evaluation. Peaks are statistically significant when exceeding the critical LOD value established (α = 0.05) and using 1000 permutations. Red line indicates the root length trait.

After performing the two analysis (IM and CIM), were recognized five putative QTLs that corresponds to associations between markers that belong to the linkage groups 1, 3, 4, 7 and 17 with root length, selected as indicative trait of resistance to aluminum toxicity (Table 3 and Figure 6).

With the saturation level of this map, it is not possible to differentiate whether the QTLs found in this preliminary study are the product of the action of one gene in that genomic region or in fact there are several genes of small effect very close among them (Flint and Mott 2001; Asins 2002), which would imply a future work in the increment of markers tested for a saturation of the map or fine mapping to molecular dissection.

It is also important to note that the results obtained in this study agree with some of the results found by Rosero (2005), after evaluating a population with similar characteristics to that used in this study and generated with a cross product of the same parents. Rosero (2005) found a QTL on linkage group 7, which explained 13.4% of the phenotypic variation related to the length of the root. In this study we found a QTL on linkage group 7, explaining 11% of the phenotypic variation for the same property. However, the findings should be confirmed, because if the characteristic root length heritability is low, some of the QTLs found may be result of the interaction between genotype and environmental conditions.

Table 3. Putative QTLs detected for root length (RL), by Interval mapping and Composite interval mapping in a F_1 population generated from the cross between *Bd* x *Br*.

	Interval Mapping analysis					**Composite Interval Mapping analysis**				
LG	**Fraction of variance explained[a] (%)**	**LOD score[b]**	**Flanking markers**	**R^2**	**QTL length cM[c]**	**Fraction of variance explained (%)**	**LOD score**	**Flanking markers**	**R^2**	**QTL length cM**
1	5,1	1,1	Apo6a /rApo8	0,4	29.3	6,6	1,4	Apo6a /rApo8	0,38	29,3
3	5,5	1,2	AC3D8/AC2D10	0,9	14,3	7,8	1,7	AC3D8/AC2D10	0,98	14,3
4	10,51	2,3	AC1D26/AC6D25	0,2	10,6	11	2,3	AC1D26/AC6D25	0,7	24,4
7	3,63	0,8	rAC1D20/A11D17	0,7	24,2	13	2,8	rAC9D16/rAC5D21	0,6	24,2
17	6,25	1,4	A12D1/rA10D17	0,4	13,2	21	4,5	A12D1/rA10D17	0,99	13,2

[a]Portion of phenotypic variation explained by the QTL.
[b]Maximum LOD score (likelihood odds ratio)
[c]The map distance between the two markers flanking the QTL.

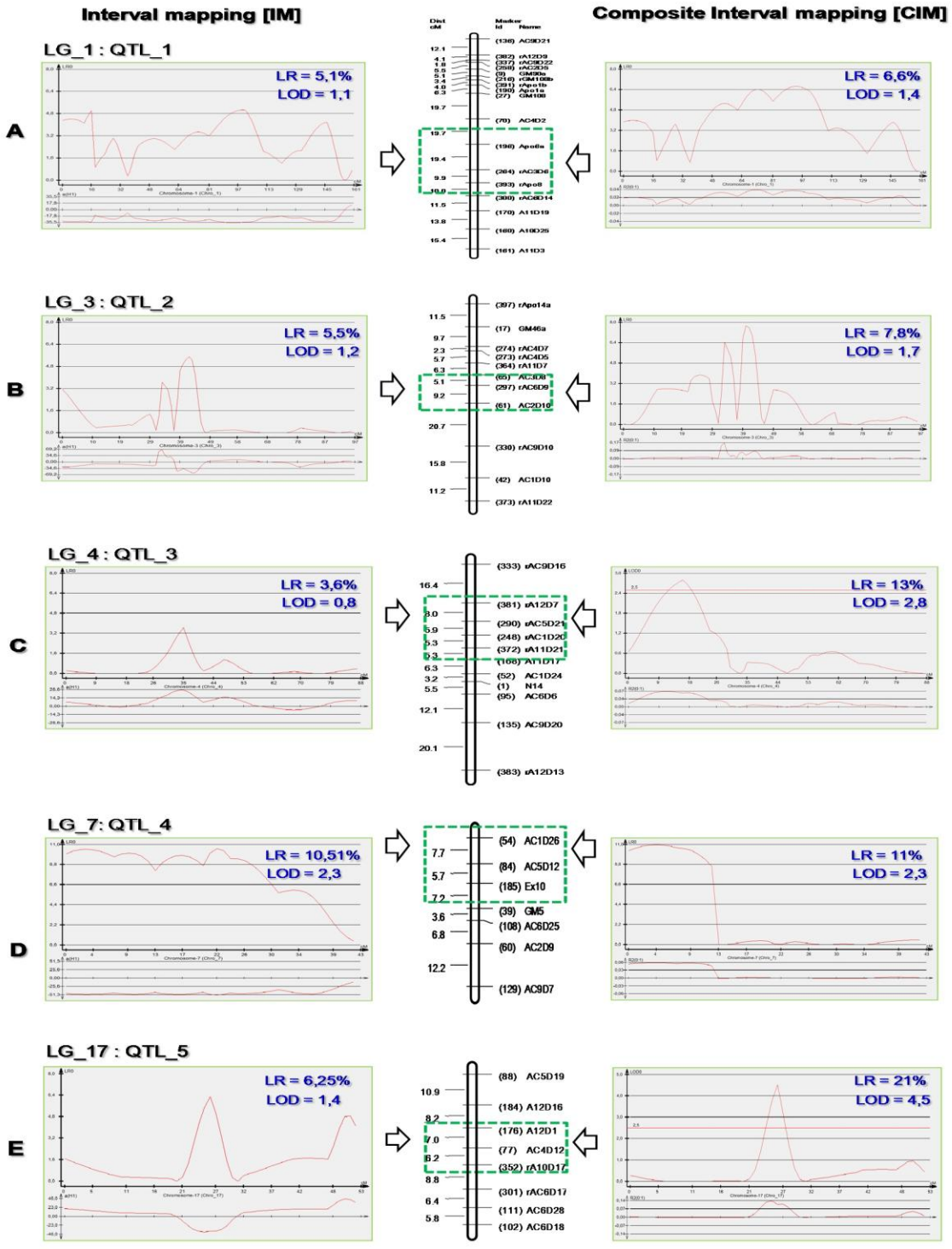

Figure 6. Linkage group locations of putative QTLs controlling root length and aluminum resistance are indicated by green rectangles and arrows. The position and statistics of each specific QTL are indicated in rows (A, B, C, D, E and F), the left column corresponds to IM and right to CIM analysis.

Comparison of QTLs for Al tolerance among related species

Major loci and QTLs controlling Al resistance have been identified in different crops, some of them related with *Brachiaria* genus. Several examples are soybean (Bianchi-Hall *et al*, 2000), sorghum (Magalhaes *et al*, 2007), maize (Ninamango-Cardenas *et al,* 2003), wheat (Raman *et al,* 2005), barley (Wang *et al*, 2007) and rice (Nguyen *et al*, 2002; Nguyen *et al*, 2003; Xue *et al,* 2007).

Comparative mapping has indicated possible homologies between Al tolerance loci in different cereal species (Kochian *et al*, 2005) and showing a high correlation with the linkage groups where QTLs were identified. The relative correspondence among the results obtained in this study and other studies that were developed in related species is showed in the Figure 7 and with special emphasis in the QTL founded on linkage groups 1, 3 and 4 reported in this preliminary study for *Brachiaria.*

QTLs found in these crops are generally associated with chromosomes 1, 3, 4 and 9. In rice for example twenty-seven QTLs important for Al tolerance, as estimated by relative root growth, were identified in five different studies and one region on chromosome 1 was held in common between all five studies. Three other chromosomal regions (chromosomes 2, 3 and 9) were identified as important in three of the five mapping population (Kochian *et al*, 2004).

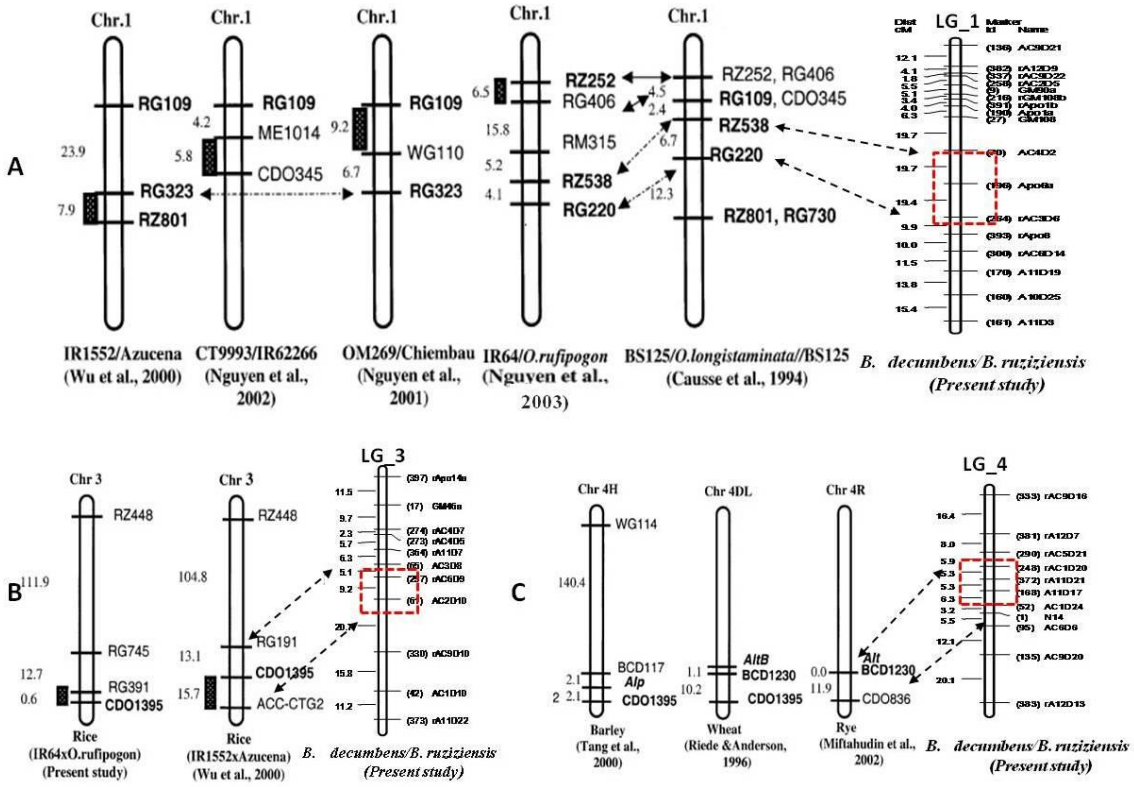

Figure 7. Comparison of QTL controlling Al resistance across different crops. **A**. Relative position of the QTLs in the chromosome 1 of different genetic backgrounds of rice respect to the QTL obtained in the present study. **B** and **C.** Comparison of QTLs controlling Al resistance across cereal crops and relative position respect to the putative QTLs of *Brachiaria.* (Source: Nguyen *et al*, 2003)

Conclusions

- A genetic linkage map was constructed for *Brachiaria decumbens,* using a hybrid population generated for the cross of *Brachiaria* with eighteen linkage groups that covering 1094 cM (mean 60.7 Cm and 6.7 markers per group) and 122 molecular markers including SSR, EST, AFLP and SCARs.

- Five putative QTLs were found in five different linkage groups (LG1, LG3, LG4, LG7 and LG17); these QTLs explained the 6.6%, 7.8%, 11%, 13% and 21% of the aluminum stress response respectively.
- It is necessary to continue with the saturation of the linkage map to increase the power of detection of genome regions that govern the quantitative characteristics of aluminum stress.
- Additional investigations into the physiological mechanisms, pathways interactions, systems biology and detailed analysis of specific genes controlling Al resistance in *Brachiaria* and related species will increase for our understanding of the evolutionary molecular genetics and the interaction of Al resistance mechanisms.

Literature Cited

Arango, A. V. 2004. Identificación de secuencias reguladoras de genes candidatos que confieren resistencia a la toxicidad por aluminio en *Brachiaria decumbens* Stapf cv. Basilisk. Universidad Nacional de Colombia. Facultad de Agronomía. Tesis de Maestría.

Asins, M. 2002. Present and future of quantitative trait locus analysis in plant breeding. Plant Breeding. 121.281:291.

Buchanan, B., Gruissen, W., and Jones, R. 2000. Biochemistry and molecular biology of plants. *American Society of Plant Physiology.*

Buitrago, M. 2003. Evaluación de los componentes fisiológicos de la adaptación a suelos acidos en una población de hubridos de Brachiaria decumbens x Brachiaria ruziziensis. Tesis pregrado. Universidad del Valle. Cali-Colombia.

Casteblanco, W., and Fregene, M. 2006. SSCP-SNP-based conserved ortholog set (COS) markers for comparative genomics in cassava (*Manihot esculenta* Crantz). *Plant Molecular Biology Reporter.* 24: 229-236.

Ciamporova, M. 2000. Diverse responses of root cell structure to aluminum stress. *Plant and Soil.* 226:113-116.

Cochrane T. T. 1991. Understanding and managing acid soils of tropical South America. In: Detruck, P., and F. N. Ponnamperuma (eds.). Rice production and acid soils of the tropics. Institute of fundamental studies, Kandy, Sri Lanka., pp. 113-122.

Flint, J., and Mott, R.2001. Finding the molecular basis of quantitative traits: successes and pitfalls. Nature reviews, Genetics, 2:437-445.

Keller-Grein G., B. Mass and J. Hanson. 1998. Variacion natural en Brachiaria y bancos de germoplasma existentes. In: Miles, J. W., B. L. Mass and C. B. do Valle (eds.). 1998. Brachiaria: Biology, agronomy and improvement. Cali, International Center for Tropical Agriculture, CIAT. 288 p.

Kochian, L. V., Hoekenga, O. A., and Piñeros, M. A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. *Annu. Rev. Plant. Biol.* 55:459-493.

Kochian, L. V., Piñeros, M. A., and Hoekenga, O. A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil.* 274:175-195.

Ikeda, H., and Tadano, T. 1993. Ultrastructural changes of the root tip cells in barley induced by comparatively low concentrations of aluminium. *Soil Sci. Plant Nutr*. 39, 109-117.

Kosambi, D. D. 1944. The stimation of map distance from recombination values. *Ann Eugen*. 12:172-175.

Lander, E., and Botstein, D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage map. Genetics, 121:185-199.

Landers E. S., P Green, J Abrahamson, A Barlow, M J Daly, S E Lincoln, L Newburg. 1987. MAPMAKER: an interactive computer program for constructing genetic linkage maps of experimental and natural populations. Genomics, 1:174-181.

Magalhaes, J. V., Liu, J., Guimaraes, C. T., Lana, U.G.P., Alves, V. M. C., Wang, Y. H., Schaffert, R. E., Hoekenga, O. A., Piñeros, M. A., Shaff, J. E., et al (2007). A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nat. Gentet.* 39:1156-1161.

Mejia, S. L. 2007. Caracterización de dos gramíneas forrajeras de *Brachiaria* (*B. decumbens* y *B. ruziziensis*) y sus recombinantes genéticos por su adaptación a suelos con bajo fosforo disponible y alta saturación de aluminio. Universidad Nacional de Colombia. Escuela de Postgrados.

Miles, J. W., B. L. Mass and C. B. do Valle (eds.). 1998. Brachiaria: Biology, agronomy and improvement. Cali, International Center for Tropical Agriculture, CIAT. 288 p.

Nimango-Cardenas, F. E., Guimaraes, C. T., Martins, P. R., Parentoni, S. N., Carneiro, N. P., Lopes, M. A., Moro, J. R., and Paiva, E. 2003. Mapping QTLs for aluminum tolerance in maize. Euphytica. 130:223-232.

Nguyen, B. D., Darshan, S. .B., Bui, B. C., Nguyen, T. V., Pham, L. N., and Nguyen, H. T. 2003. Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (Oryza sativa L.). *Theor. Appl. Genet*. 106:583-593.

Pizarro E. A., C. B. do Valle, G. Keller-Grein, R. Shultze-Kraft and A. H. Zimmer. 1998. Regionales experience with Brachiaria: tropical America – savannas, p. 225-243. In J. W. Miles, B. L. Mass and C. B. do Valle (eds.). 1998. Brachiaria: Biology, agronomy and improvement. Cali, International Center for Tropical Agriculture, CIAT. 288 p.

Raman, H., Zhang, K. R., Cakir, M., Appels, R., Garvin, D. F., Maron, L. G., Kochian, I. V., Moroni, J. S., Raman, R., Imtiaz, M., et al. 2005. Molecular characterization and mapping of *ALMT1*, the aluminum-tolerance gene of bread wheat (*Triticum aestivum* L.), *Genome*. 48:781-791.

Rao I. M., Kerridge P. C., Macedo M.C.M. 1996. Nutritional requirements of Brachiaria and adaptation to acid soils. In: J. W. Miles, B. L. Maass and C. B. do Valle (eds.), 1998. Brachiaria: Biology, agronomy and improvement. Cali, International Center for Tropical Agriculture (CIAT), 288 p.

Sturr WW. Hopkinson JH, Chen CP. 1998. Experiencia regional con *Brachiaria*: Asia, el Pacifico Sur y Australia. En: Miles, J., B. Mass, & C. do Valle. 1998. *Brachiaria:* Biología, Agronomía y Mejoramiento. CIAT, Palmira, Colombia. p. 282-296.

Swenne, A., Louant, B.P., and Dujardin, M. 1981. Induction par la colchicine des formes autotetraploides chez *Brachiaria ruziziensis* German et Evrard (Graminée). *Agronomie Tropical,* 36 (2): 134 – 141.

Wang, S. C., Basten, and Zeng, Z. B. 2005. Windows QTL Cartographer 2.5. Department os statistic, North Carolina State University. Raleigh, NC.

Wang, J. P., Raman, H., Zhou, M. X., Ryan, P. R., Delhaize, E., Hebb, D. M., Coombes, N, and Mendham, N. 2007. High-resolution mapping of the Alp locus and identification of a candidate gene HvMATE controlling aluminum tolerance in barley (Hordeum vulgare L.). Theor. Appl. Genet. 115:265-276.

Wu K., W. Burnquist, M. Zorreéis, T. Tew, P. Moore, and S. Tanksley. 1992. The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor., and Applic. Genetics, 83:294-300.

Xue, Y., Jiang, L, Su, N., Wang, J. K., Deng, P., Ma, J. F., Zhai, H. Q., and Wan, J. M. 2007. The genetic basis and fine-mapping of a stable quantitative-trait loci for aluminium tolerance in rice. *Planta.*227.

ANNEX 4

Project Title

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

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Improving genetic adaptation

ANNEX-4

Improving genetic adaptation

Output 4: *Genetic adaptation improved of common bean and Brachiaria to drought and aluminum toxicity, through deployment of phenotypic screening methods to develop DNA markers*

1. Yield of elite lines derived from intraspecific and interspecific crosses in response to drought and acid soil complex

Contributors: S. Beebe, I. Rao, L. Butare, C. Cajiao, J. Polanía, J. Ricaurte, and M.A. Grajales

Rationale: Moisture deficits, poor soil fertility and an acid soil complex are the most common abiotic stresses faced by resource-poor smallholder farmers in the tropics. Genetic resistance to these stresses, when available, remains the most practical and economically viable solution. However, abiotic stresses seldom occur independently in farmers' plots in the developing world. Since aluminum toxicity can limit root growth and root penetration in acid soils, it can also exacerbate moisture deficits by reducing access to water in lower soil strata. A central issue addressed in the current project was to obtain germplasm of common bean with multiple resistance to abiotic stress factors, specifically drought and aluminum toxicity. Significant progress was made in a previous project on improving bean for drought resistance, while improvement in aluminum tolerance was modest using intraspecific crosses. The secondary gene pool of common bean is composed of species that evolved in humid environments, and often on acid volcanic soils with potential for aluminum toxicity. These species can be crossed with relative ease to common bean, although the agronomic quality of the progenies is usually quite poor. Therefore it was decided to seek sources of Al resistance in the secondary gene pool, and to use such sources to improve tolerance of common bean to Al, in combination with drought resistance.

Methods: In a previous project, genotypes from the Andean gene pool of common bean had expressed what appeared to be physiological resistance to Al toxicity, while genotypes of the Mesoamerican pool appeared to derive their resistance from an escape mechanism of shallow rooting. Lines derived from crosses among these two gene pools were advanced and tested for their yield potential in a field with Al toxic acid soil in Santander de Quilichao, Colombia. In 2006A season, 100 lines and checks were planted in a 10 x 10 lattice design and evaluated for grain yield. In the 2006B season selected lines from the previous season were selected for additional testing, and these were arranged in three 4 x 4 lattice designs to try to control better the spatial variability of the field trials.

A collection of 147 accessions of *Phaseolus coccineus* and *P. polyanthus* were planted for an unreplicated screening on an Al-toxic, acid oxisol. Several accessions were selected for vegetative vigor, and after additional evaluations in greenhouse trials, especially in solution culture, 3 accessions of *P. coccineus* were identified for crossing (G35066, G35346, G35464), one accession of which was represented by two individual plant selections (G35346-2Q and G35346-3Q). Simple cross F1s with drought resistant common bean SER 16 were backcrossed to SER 16. SER 16 has excellent remobilization of photosynthate to grain under drought and other stressful conditions. Field observations on F2 populations suggested that the population of SER 16 x (SER 16 x G35346-3Q) was highly variable in response to Al and possessed some families with excellent Al resistance. This population was therefore chosen for advancing by Single Seed Descent to F5-derived Recombinant Inbred Lines (RIL), while the same population was selected in parallel fashion by pedigree selection under Al-toxic field conditions based on vegetative vigor and productivity. Ninety-six RIL were evaluated in yield trials with 3 replications, as were lines selected by pedigree selection.

Yield trials of RILs were carried out under three conditions: acid soil complex, drought, and unstressed irrigated. Trials in the acid soil complex were carried out at Santander de Quilichao, Colombia (996 m above sea level; 26^{o}C average temperature; oxisol) in 2008 and 2009. Plots were 2 rows by 3.7 m long, and were irrigated as necessary. The breeding line VAX 1 was used an Al-tolerant check and as a benchmark of past progress. Drought plots and unstressed irrigated plots (2 rows by 5 m long) were established at CIAT headquarters near Palmira, Colombia (1000 masl; 26^{o}C average temperature; Molisol) in the dry season between June and September in 2008 under intermittent drought and in 2009 under terminal drought. Grain yield was taken at maturity. Additionally, 100 lines developed by pedigree selection were yield tested in 2008 (intermittent drought).

Results: *Yield of intraspecific lines in an acid soil*: In 2006 acid soil stress was severe and all checks yielded less that 400 kg/ha (Table 1). Among lines tested, the VAX 1 check was outyielded by as much as 220 kg/ha. The cross 14586 produced a number of advanced lines, and in several seasons at least one of these is among the best in yield. In the second season of 2007 the same pattern was observed (Table 2), in which four sister lines of this cross outyielded VAX 1 by as much as 600 kg/ha. Although very encouraging that superior materials had been derived in crosses among the two major gene pools of common bean, no single line expressed a stable reaction over years, possibly due to the difficulty in obtaining uniform field conditions for yield evaluation.

Table 1. Best intraspecific lines drawn from 100 entries tested in a lattice design on an Al-toxic acid oxisol, 2006

Cross code	Pedigree	Days to Maturity	Yield (kg/ha)
14586- 1	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-15Q-MQ-MQ-12Q-MC	70	588
14300-22	G 24601 x (G 22041 x BRB 198)F2/-MQ-7Q-2Q-MQ-MQ-7Q-MC	69	570
14304- 2	RAB 655 X (MAM 49 X RIB 66)F1/-MQ-MQ-MQ-8Q-MQ-MQ-14Q-MC	68	556
14894-21	(RAB 655xG 19168)F1x(MAM 49xG 21212)F1/-MQ-MQ-3Q-MC	68	532
14889- 1	(MAM 38xG 21212)F1x(SEA 15xG 21212)F1/-MQ-MQ-6Q-MC	68	531
14713- 7	(MAM 38xG 21212)F1 X (G 24601x(G 22041xBRB 198)F2)F1/-1Q-MQ-MQ-13Q-MC	69	510
	VAX 1 (Res. Check)	69	366
	Carioca (Comm. Check)	63	313
	Tio Canela (Comm. Check)	67	361
LSD (0.05)			190

Table 2. Best intraspecific lines drawn from 16 entries tested in a lattice design on an Al-toxic acid oxisol, 2007

Cross code	Pedigree	Days to Maturity	Yield (kg/ha)
14586- 1	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-15Q-MQ-MQ-18Q-MC-MQ-MQ	72	1563
14586- 1	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-15Q-MQ-MQ-10Q-MC-MQ-MQ	72	1392
14586- 1	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-15Q-MQ-MQ-12Q-MC-MQ-MQ	72	1385
14586- 1	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-15Q-MQ-MQ-2Q-MC-MQ-MQ	73	1247
14586- 2	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-3Q-MQ-MQ-10Q-MC-MQ-MQ	70	1222
14586- 1	((G 24601x(MAM 38xBRB 198)F1)F1xG 11015)F1 X (MAM 38xG 21212)F1/-MQ-15Q-MQ-MQ-17Q-MC-MQ-MQ	72	1205
	VAX 1 (Res. Check)	69	917
	Tio Canela (Comm. Check)	63	824
LSD (0.05)			443

Yield of interspecific RILs in several environments: In Table 3 are presented yield data of Recombinant Inbred Lines (RILs) among parents that combine traits of drought resistance and tolerance to toxic Al. Data are presented on selected lines that showed particular promise across environments. Besides the standard checks for Al tolerance (VAX 1) and drought (SER 16), the line ALB 252 is included as representative of promising intraspecific progenies. A few of the RILs such as ALB 6 expressed superior yield potential across environments. ALB 6 was the best yielding under acid soil stress and yielded marginally more than any of the checks in all environments except for VAX 1 under irrigation. ALB 91 was the second best across environments and was also identified in greenhouse soil tube trials as having excellent root penetration in acid soil. SER 16 was surprisingly good in all trials, reflecting the value of its excellent photosynthate remobilization for yield in multiple environments. ALB 252 performed comparably to the VAX 1 check in acid soil, but was poor across environments. In general, the agronomic quality of the elite interspecific progenies was superior to ALB 252 and other intraspecific lines, due to the contribution of SER 16 to the interspecific progenies.

Table 3. Superior interspecific RILs derived from SER 16 and *Phaseolus coccineus* by single seed descent, tested under acid soil complex (2008), intermittent drought (2008), terminal drought (2009), and irrigated non-stress conditions (2008).

Line	Al (kg/ha)	Intermittent drought (kg/ha)	Terminal drought (kg/ha)	Irrigated (kg/ha)	Ave. (kg/ha)
ALB 6	907	2513	1985	3607	2253
ALB 91	735	2074	2275	3267	2088
ALB 58	727	2134	1645	3191	1924
ALB 60	791	1749	1604	3539	1921
ALB 252*	647	1822	1140	2792	1601
SER 16 (Ch, Drt)	647	2364	1764	3339	2028
VAX 1 (Ch.-Al)	649	1383	1421	3705	1790
LSD (0.05)	285	490	381	474	

* Intraspecific line used as check

Yield of interspecific elite lines in drought and acid soil. Results with lines subjected to intensive selection were comparable to results with RILs in terms of the range of yield values under acid soil stress. However, the number of superior lines was greater, due in large part to the inclusion of several elite sister lines. A greater advantage of directed selection might be seen under intermittent drought in 2008, where some lines exceeded the yield of the drought parent SER 16 by 500-600 kg/ha. An especially promising line, ALB 213, displays excellent agronomic qualities of plant type, uniformity of maturation, and productivity (Figure 1). It has proven to be an excellent parent in subsequent crosses.

Table 4. Interspecific lines derived from SER 16 and *Phaseolus coccineus*, selected by pedigree selection under Al toxic acid soil and intermittent drought, with checks.

Line	Days to maturity	Al (kg/ha)	Intermittent drought (kg/ha)
ALB 204	68	906	2781
ALB 211	65	896	2563
ALB 208	66	877	2625
ALB 215	65	859	2722
ALB 214	63	846	2879
ALB 213	67	755	3029
ALB 205	68	656	3129
Checks			
SER 16 (Ch., drt)	63	742	2520
VAX 1 (Ch., Al)	68	560	2633
G21212	68	883	2683
ALB 252*	70	882	2824
LSD (0.05)		231	568

*Intraspecific line used as check

Figure 1. An elite line developed from an interspecific cross of (SER 16 x (SER 16 x G35345-3Q) under intermittent drought stress in 2008.

Discussion: A few lines presented statistically significant differences above the yields of the respective checks in drought (Tables 3 and 4) and acid soil (Table 1, 2 and 4). However, many more lines yielded marginally more than the checks, and it can be stated with confidence that these are as good as the checks in those conditions. This in itself is not trivial, when the lines combine resistance to two stresses. Such lines were registered with both intraspecific and interspecific crosses in tolerance to an acid soil complex in which Al toxicity played a dominant role. However, the interspecific crosses displayed both wider adaptation, including drought resistance, and better agronomic qualities beyond yield per se at the acid soil site. Most intraspecific progenies displayed an undesirable plant type, resulting from the wide inter-gene pool crosses from which they were derived. One would expect equally bad plant habit from the interspecific crosses, but in fact many of the interspecific lines were upright in habit, with good to excellent yield potential. This advantage is attributed in part to the value of SER 16 as an unusually good parent characterized by excellent photosynthate remobilization.

However, the *Phaseolus coccineus* parent also contributed traits for yield improvement, both under acid soil and probably in unstressed irrigated conditions and drought. This contribution may be in two interrelated ways: through a longer growth cycle that permits more biomass accumulation, and through specific root traits. Although the best yielding lines were in fact later maturing than SER 16, the difference was modest (4-5 days), and lines matured well within an acceptable range of maturity. As to specific root traits that might be derived from *P. coccineus*, the physiological analysis of roots demonstrated that there is great variability among lines, and some of these are likely contributing to yield. As noted, ALB 91 was one of the most stable and high yielding lines, and it displays a root system that penetrates well into acid soil. However, no RILs were as good as the coccineus parent when analyzed for root traits, and it may be necessary to cross among the interspecific progenies to recover more of the coccineus phenotype. Other root traits remain to be studied, such as the capacity to penetrate compacted soil.

The success in combining multiple stress resistance bodes well for breeding common bean for wider adaptation, especially as climate change may create more environments in which abiotic stress becomes more acute. *P. coccineus* has proven to be an important resource in this regard, and should be studied more amply. In the past it has been little utilized beyond its role as a source of disease resistance, and even in this case, with little success. The key to success in the current study seems to have been

crossing to a common bean genotype, SER 16, with enhanced capacity of photosynthate remobilization to grain. This capacity appears to "tame" the overly vegetative growth pattern of coccineus, resulting in agronomically useful progenies.

Conclusions: Substantial yield gain was made in tolerance to Al toxicity over the VAX 1 check, and in multiple stress resistance. Unexpectedly, the interspecific crosses produced progenies with excellent agronomic traits, and unusually good yield potential. We believe that this reflects the enhanced photosynthate remobilization of SER 16 in combination with biomass accumulation and root traits derived from *Phaseolus coccineus*.

2. New sources of Resistance in *Phaseolus* species to Individual and Combined Stress Factors of aluminum-toxic acid soil and Drought

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Abbreviations: HAl, high aluminum soil saturation; LA, leaf area; LAl, low aluminum soil saturation; MRD, mean root diameter; PC, percent of control; RDBW, root dry biomass weight; RDP, rooting depth; REGWQ, Ryan-Einot-Gabriel-Welsh Multiple Test; R:S ratio, root to shoot ratio; RTP, number of root tips; SDBW, shoot dry biomass weight; SRL, specific root length; TRER 48h, tap root elongation rate between 0 to 48 hours of exposure to with and without aluminum in solution; TRER 5d, tap root elongation rate between 0 to 5 days of exposure to with and without aluminum in solution; TRL, total root length; WS, water stress; WW, well watered.

Abstract: Improved screening methods and sources of resistance would speed progress in breeding for abiotic stress resistance in common bean. Bean species and genotypes show wide phenotypic variability in resistance to aluminum (Al) and drought stress. The objective of this study was to identify sources of resistance among six genotypes of *Phaseolus vulgaris*, four of *P. coccineus*, and one of *P. acutifolius*, to Al and drought stress using hydroponic and soil tube screening methods. A hydroponic screening of Al resistance was carried out using a basal nutrient solution with and without 20 µM Al. Two experiments in 80 cm long soil tubes were carried out using an oxisol with 76% (pH = 4.1) and 83% (pH = 4.14) Al saturation, for topsoil and subsoil, respectively. The three experiments showed an average of 36.9 to 53.5% inhibition of root growth between high and low Al treatments. Differences in root development and distribution were observed. Two accessions of *P. coccineus* (G35346-2Q, G35464-5Q) and one Andean genotype (ICA Quimbaya) were outstanding in root and shoot growth in the high Al treatments. *P. coccineus* accession (G35346-3Q) was outstanding under combined stress of drought and acid soil. The methodology used to evaluate genotypes for individual and combined stress factors served to identify superior parental genotypes for use in breeding programs.

Introduction: Common Bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes for direct human consumption in the world. The crop is mainly produced on small-scale farms in developing countries in Latin America and Africa where both biotic and abiotic stress factors limit production. About 60% of the bean growing area is affected by drought while about 40% of the bean growing area is affected by aluminum (Al) toxicity, resulting in yield reductions from 30% to 60% (Wortman et al., 1998; Thung and Rao, 1999). Abiotic stress resistance is by its nature more complex physiologically, typically subject to large environmental effects and has been less well studied than biotic stress resistance in common bean (Rao, 2001).

Aluminum is one of the most abundant minerals in the soil comprising approximately 7% of soil mass. At neutral or weakly acidic pH, it exists in insoluble forms of alumino-silicate or oxide; however, in an acidic soil, it is solubilised into a phytotoxic form. Toxic Al levels damage roots, restrict plant size, and lower yield in most crops (Villagarcia et al., 2001). Root stunting is a consequence of Al-induced inhibition of root elongation (Mossor-Pietraczewska, 2001). The toxic effects of Al in soil can be overcome by adding appropriate amendments such as lime (Pandey et al., 1994; Villagarcia et al., 2001). But lime application is a short term solution and not affordable to most households in developing countries that grow beans. Developing bean genotypes tolerant to acid soil conditions is an ecologically friendly, energy-conserving, and economical solution for resource-poor farmers in the tropics (Rangel et al., 2005).

Differential genotypic response to Al stress contributes to identification of new sources of Al resistance as well as improved understanding of mechanisms of Al resistance in common bean (Rangel et al., 2005; 2007; 2010). Significant genotypic differences in Al resistance in common bean were reported based on Al-inhibited root elongation in nutrient solution (Foy, 1988; Massot et al., 1999; Rangel et al., 2005; Manrique et al., 2006; Rangel et al., 2007). The understanding of Al resistance in plants has been limited

by inadequate screening methodologies (Villagarcia et *al*., 2001). Screening for Al resistance based on field data would be strengthened and complimented by evaluation of specific phenotypic and physiological traits. Hydroponic systems have been used with success in evaluation of Al resistance for many crops, and are an attractive alternative. They allow evaluation of a large number of genotypes quickly and have been used to identify parental stock for soybean breeding (Campbell and Carter, 1990; Carter and Rufty, 1993; Spehar, 1994; Bianchi-Hall et al., 1998; Bianchi-Hall et al., 2000; Silva et al., 2001). Hydroponic systems for evaluation of genetic materials provide a strict control of nutrient availability and are widely used in genetic studies. To complement the hydroponic screening system, researchers at CIAT have developed a greenhouse screening method using vertical soil tubes with high Al saturation to quantify genotypic differences in root development and distribution in Al-toxic soil conditions (CIAT, 2008). Soil-based systems offer a medium that is more similar to field conditions. This methodology also permits characterization of the root system, in terms of rooting depth and branching of fine roots in a soil with known bulk density.

Drought is a major abiotic stress in many parts of the world (Johansen et al., 1994). There is an urgent need for developing high yielding drought resistant cultivars that use water efficiently, reduce dependence on irrigation water and associated production costs, increase and stabilize yield in drought-prone environments, and increase profit margins for producers (Muñoz-Perea et al., 2006)**.** Selection for drought resistance based on yield alone may not bring the required genetic shift in specific physiological attributes as different mechanisms would have different opportunities for expression under different conditions (Subbarao et al., 1995). Rooting pattern, especially greater root length in lower soil strata, is an important drought resistance mechanism for common bean (Sponchiado et al., 1989). Drought tolerant bean genotypes could extend their roots to 1.2 m depth in drought environments, whereas the sensitive genotypes could not extend their roots beyond 0.8 m; and these differences in rooting depths were reflected in overall shoot growth and yield (White and Castillo 1988). Root systems show considerable architectural variation among species, among genotypes of given species, and even with different parts of a single root system (Lynch, 1995). Wild relatives in many legumes possess deep rooting capability that could be transferred to cultivated legumes. A number of *Phaseolus* species, such as *P. acutifolius*, *P. retensis*, and *P. coccineus*, have deep and/or tuberous primary root attributes (Singh and White 1988).

Understanding the genetic and physiological mechanisms by which plants cope with changes in environmental conditions is critical for creating efficient strategies to develop stress-resistant cultivars for sustainable production systems (Rao, 2001). Abiotic stress factors often co-occur in farmers' fields. Roots that are stunted by Al toxicity are inefficient in absorbing both nutrients and water (Mossor-Pietraczewska, 2001). Al-tolerant plants may be more drought tolerant and require lower inputs of lime and P fertilizer than less tolerant genotypes (Little, 1988).

Improving resistance to two complex stresses such as Al and drought in common bean requires identifying new sources of resistance among *P. vulgaris* accessions and in sister species including *P. coccineus* and *P. acutifolius*. The objective of this work was to identify potential parents based on phenotypic differences among bean genotypes in root development and root distribution under individual and combined stress factors of high Al toxicity and drought.

Materials and Methods. Three greenhouse trials were conducted at CIAT headquarters in Palmira (Lat. 3°29'N; Long.76°21'W, Altitude 965 m) using hydroponic and soil tube systems. For hydroponic screening a low ionic strength nutrient solution was used to evaluate root traits of seedlings grown with or without 20 µM Al in a basal nutrient solution (Rangel et al., 2005, 2007). For purposes of this study, "Al resistance" refers to the response of a genotype to toxic Al in the hydroponic system. Tolerance to Al-toxic acid soil conditions refers to tolerance to high Al saturation in acid soil together with low availability of nutrients. Evaluation in soil tubes for Al-toxic acid soil tolerance was done using an oxisol, collected from Santander of Quilichao (Department of Cauca, Colombia), with 1.1 g cm^{-3} bulk density. A second soil tube trial was conducted for individual and combined stress of acid soil (highly Al-saturated soil) and drought using the same oxisol from Quilichao. The experimental design was a randomized complete block with three replications for both soil tube experiments and 5 to 10 replications for hydroponic evaluation. In the first soil tube experiment response to only acid soil Al stress was evaluated, whereas in the second soil tube trial four treatments were applied in a factorial design: well watered soil tubes or drought induced stress under high or low acid soil Al stress conditions.

Plant Materials: Eleven bean genotypes were selected for the study, including 4 *P. coccineus* accessions (G35066-1Q, G35346-2Q, G35346-3Q and G35464-5Q); 6 common bean genotypes including 4 lines of the Mesoamerican gene pool (VAX1, VAX3, VAX6, SER16) and 2 large seeded

beans of the Andean gene pool (ICA Quimbaya and IJR, Indeterminate Jamaica Red); and one *P. acutifolus* accession (G40159). The *P. coccineus* accessions had been identified in a field screening of 155 entries of *P. coccineus* and *P. polyanthus* in an Al toxic field site in Santander of Quilichao, Colombia, based on shoot vigor. *P. acutifolius* is a drought resistant desert species and one of its accessions, G40159 had been identified as especially drought tolerant. The VAX lines had been selected for common bacterial blight resistance in Santander of Quilichao during their development, and VAX1 had expressed good shoot vigor in Al toxic soils. These plant materials were evaluated for their phenotypic differences under individual and combined stresses of Al and drought. Seeds were surface sterilized with sodium hypochlorite (1% for 5 minutes) and washed with abundant deionised water. Seeds were germinated on filter paper for 2 to 3 days before planting in soil tubes. For hydroponic experiments, germinated seeds were transferred to small pots containing sterile sand for root development, and were carefully removed from the sand after 3 days. Seedlings with uniform vigour and tap root length were chosen for evaluation in hydroponic system.

Evaluation for Al resistance Using Hydroponic System: The hydroponic experiment was conducted during November and December 2007. Plants were grown in a greenhouse with an average temperature of 31.1/22.3°C (day/night), a relative humidity of 48.0/67.3% (day/night), and with a maximum photosynthetic active radiation of 1100 µmol m^{-2} s^{-1} photon flux density at noon. Seedlings with uniform root length (5-7 cm) were selected for evaluation with nutrient solution composed with 5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 at pH 4.5. Twenty liter plastic tanks were filled with 16 liters of nutrient solution. Each seedling was placed in an individual compartment in a tray floating on the solution and the nutrient solution was permanently aerated with a compressor during the evaluation. Acclimation to low pH before applying Al treatment was made by adjusting the initial solution pH to 5.5, followed by pH 4.9 for 18 hours and lastly by pH 4.5 for 24 hours. New nutrient solutions were prepared in two sets of tanks (16 l each), one containing only nutrient solution and the other in which was added 20 µM $AlCl_3$. Plants with same root length were distributed in pairs in each treatment after measuring the length of tap root with a ruler. Solutions were renewed every third day. Root elongation at 48 hours and after 5 days under Al stress was determined based on the initial measurement of tap root length.

Root length and architecture: At harvest roots were separated from the rest of the plants, saved in plastic bags and refrigerated at 4°C while proceeding to analyze images using a flatbed colour scanner "Epson Expression1680 Scanner". Differences in root attributes among genotypes including total root length, mean root diameter, and number of root tips were analyzed using Win Rhizo® software program. Root elongation rate, inhibition of tap root growth and specific root length (root length per unit dry weight) were calculated; and the root dry weight was determined by drying roots at 65°C in an oven for 48 h. Difference between the initial and final tap root length during the treatment period was defined as root elongation rate (RER); and aluminum-induced inhibition of root elongation calculated as Rangel et al. (2005):

$$\text{Inhibition of root elongation (\%)} = \frac{RER_{control} - RER_{Al}}{RER_{control}} \times 100$$

Evaluation for Individual and Combined Stress Factors of Al-toxic Soil and Drought

Simulating the stress of Al-toxic acid soil: Soil for this experiment was collected from Santander of Quilichao, Cauca Department (3° 06' N lat., 76° 31' W long; 990 m altitude), Colombia. Soil used in the Al stress treatment (high Al) was characterized by a pH of 4.1 and 76% Al soil saturation (0-10 cm) for top-soil (top 10 cm of the cylinder) and 83% Al saturation for subsoil (10-75 cm) with pH 4.14 (Table 1). This treatment did not receive any additional fertilizer application to simulate high Al with low nutrient availability soil conditions that are typical of Al-toxic acid soils.

The soil tubes (cylinders) for low Al treatment were packed with Quilichao soil (described in Table 1), previously fertilized with adequate amendments (g.kg^{-1} soil) for top soil (0-10 cm): 6.32 N (Urea), 9.09 P (triple superphosphate), 9.09 Ca (triple superphosphate), 6.99 K (KCl), 10.91 Ca ($CaCO_3$), 0.85 Mg ($MgCO_3$ or dolomite lime), 0.15 S (elemental sulphur), 0.2 $CuCl_2.2H_2O$, 0.02 B (H_3BO_3) and 0.01 Mo ($NaMoO_4.2H_2O$); and for subsoil (10-75 cm) 14.76 N (Urea), 21.2 P (triple superphosphate), 21.21 Ca (triple superphosphate), 16.32 K (KCl), 25,45 Ca ($CaCO_3$) 1.97 Mg ($MgCO_3$ or dolomite lime), 0.36 S (elemental sulphur), 0.46 $CuCl_2.2H_2O$, 0.05 B (H_3BO_3) and 0.02 Mo ($NaMoO_4.2H_2O$). This fertilization didn't affect Al saturation and pH of the amended soil. The polyethylene cylinders were inserted into PVC

pipes and were maintained at 80% field capacity by weighing each cylinder every three days and applying water to the soil at the top (Polania et al., 2009).
This soil tube experiment was conducted during June-July 2007 in greenhouse in Palmira (CIAT/Colombia) with an average temperature of 29.4/23.1°C (day/night), relative humidity of 57.2/79.4% (day/night), and maximum photosynthetic active radiation of 1100 µmol m^{-2} s^{-1} photon flux density at noon.

Table 1. Characterization of two soils from Santander of Quilichao used for evaluating acid soil tolerance with their chemical characteristics.

Al saturation	Soil depth (cm)	pH	Al	Ca	Mg	K	Al sat.	SOM†	Available P
			(cmol kg^{-1} soil)				(%)	(%)	(mg kg^{-1})
High	0-10	4.11	4.60	0.94	0.30	0.18	76	5.96	8.80
High	10-20	4.14	4.40	0.69	0.16	0.07	83	4.94	3.30
Low	0-10	4.45	1.65	3.32	0.89	0.26	28	5.38	9.70
Low	10-20	4.29	3.02	1.63	0.25	0.28	58	4.56	4.30

† Soil organic matter

Shoot and root attributes: Total chlorophyll content (SPAD) was measured every week using SPAD-502 Chlorophyll meter (Minolta camera Co., Ltd, Japan). At the time of harvest (29 days after planting), leaf area was determined by scanning leaves of each genotype using a LI-3100 Area meter (LI-COR Biosciences). Shoot dry weight was measured after drying leaves, stems and pods in an oven at 70°C for 72 hours**.** Each soil tube was sliced into six layers representing different soil depths (0-5, 5-10, 10-20, 20-40, 40-60, 60-75 cm), soil and roots were collected, and roots washed and cleaned to separate living plant roots from organic debris before scanning. Root length and biomass distribution was determined for each profile and cutting of soil tubes at different depths did not permit measuring number of root tips.
Simulating the combined stress of Al-toxic acid soil and terminal drought: Individual and combined stress of Al and drought was evaluated in September 2008 under greenhouse conditions at an average temperature of 30.7/23.3°C (day/night), a relative humidity of 49/68.3% (day/night), and at an average photosynthetic photon flux density of 820 µmol m^{-2} s^{-1} during the day. Plants were grown in transparent tubes inserted in PVC pipes as previously described for Al screening with same soil type from Santander de Quilichao. The low Al saturation treatment was fertilized as described above. The experimental design was a randomized complete block with two levels of Al saturation in soil (high and low) and two levels of water supply (well watered and terminal drought simulation). Each cylinder was packed with two types of soil (top-soil and sub-soil); and maintained at 80% of field capacity by weighing every three days (4780 g for high Al saturation treatment tubes and 4910 g for the low saturation). Water stress was imposed in the terminal drought treatment after 10 days of initial growth while for the well watered treatment, water was applied to the top of cylinders to maintain them at 80% of field capacity. At harvest time, shoots and roots of 33 day-old plants (23 days without water application in the terminal drought treatment) were separated, and leaf area measured by scanning leaves. Shoot biomass was determined after drying leaves and stems in an oven. Roots were processed in the same way as for previous soil tube trial with Al-toxic acid soil alone and the same parameters were determined.
Statistical Analysis: Analysis of variance was performed by the ANOVA statistical procedure of SAS (SAS 9.1, 2002-2003; SAS institute Inc.; SunOS 5.9 platform). The means were compared using Ryan-Einot-Gabriel-Welsh Multiple Test. This test controls the type I experiment wise error rate. Means for each dependent variable were grouped, and means with the same letter are not significantly different statistically. Differences between genotypes were analyzed with the least significant difference (LSD). Correlation coefficients were calculated (PROC CORR) for all pairs of genotypic means across all replications with each Al treatment and for all three experiments.

Results

Plant Materials: The response of root attributes (root length, mean root diameter, specific root length and rooting depth) to Al stress was used to assess resistance of beans to Al toxicity. A significant variation ($P < 0.001$) among 11 genotypes was found in this study using both hydroponics (Table 2b) and soil tube

(Table 3b, 4b) screening systems. Relationships between traits were considered based on expectations of which combinations of traits would confer a favourable reaction to Al toxicity. High variability in root architecture was observed between genotypes except for root length at 48 h of treatment with 20µM Al in hydroponic system. Since *P. coccineus* accessions did not show any difference in the hydroponic system between treatments with and without Al in solution for 48 h, the exposure time was extended to 5 days where significant genotypic variation ($P < 0.001$) in Al resistance was observed.

Evaluation for Al Resistance Using Hydroponic System

Root architecture under Al stress: Al stress in hydroponic screening affected root growth characteristics of all 11 bean genotypes tested. Results on total root length (TRL), tap root elongation rate (TRER) at 48 h (TRER48h) and 5 days (TRER5d), root dry biomass weight (RDBW), mean root diameter (MRD), specific root length (SRL) and number of root tips (RTP) showed considerable architectural variation in response to Al stress (20µM Al) among bean species and varieties (Table 2a). Genotypic differences were highly significant ($P < 0.001$) for all root traits except for root length at 48h where the significance was at $P < 0.05$. Three *P. coccineus* accessions (G35464-5Q, G35346-2Q, and G35346-3Q) showed a high level of resistance to Al whereas three Mesoamerican bean genotypes (VAX6, VAX3 and SER16) were found more sensitive. Genotype x treatment (Level of Al) interaction was significant ($P < 0.001$) for SRL, for RTP ($P < 0.01$), and for MRD and TRL ($P < 0.05$) (Table 2b).

Total root length was highly ($P < 0.001$) correlated with tap root length after 5 days ($r = 0.92$), with root biomass dry weight ($r = 0.91$), with number of root tips ($r = 0.96$); and with tap root length at 48 h ($r = 0.77$, $P < 0.01$). Correlation was also found between TRE at 48 h and TRE at 5 d ($r = 0.74$, $P < 0.01$); and between TRE at 48 h and RTP ($r = 0.78$, $P < 0.01$) (Table 2b).

Inhibition of tap root elongation rate and changes in root morphology: Al treatment (20 µM Al) strongly affected root-elongation rate with means varying from 0.015 to 0.10 $mm.h^{-1}$. Tap root growth was inhibited by 21.3% to 60.7%, and the increase of mean root diameter ranged from 8.06 to 20.5%. The root growth rate (RGR) of three *P. coccineus* accessions (G35464-5Q, G35346-2Q and G35346-3Q) and ICA Quimbaya (an Andean genotype) was high compared to other genotypes (rate > 0.06 $mm.h^{-1}$), with no significant difference between the four genotypes. Among these Al resistant bean genotypes, G35464-5Q and G35346-2Q showed significant differences at $P < 0.05$ (REGWQ test) with two susceptible genotypes (VAX1 and VAX6) (Figure 1a). After 5 days of Al exposure, root growth was inhibited by 42.7%. Three genotypes (VAX3, VAX1 and G35066-1Q) were sensitive to Al with an inhibition superior to 50%; six genotypes including SER16, Indeterminate Jamaica Red (IJR), G35346-3Q, VAX6, ICA Quimbaya, and G40159 were intermediate with a root growth inhibition ranging between 37.5% and 44.0%, whereas only two *P. coccineus* accessions (G35346-2Q and G35464-5Q) were resistant with a root growth inhibition of 30.8% and 21.3%, respectively (Figure 1b). These two genotypes were significantly different at $P < 0.05$ (REGWQ test) from sensitive lines VAX3, VAX1 and G35066-1Q (Figure 1b). Seedlings grown in presence or absence of Al (0 and 20 µM Al) after 5 days also showed differential response among genotypes to Al for the increase of root diameter (Figure 1c). Three genotypes (VAX6, G35346-3Q, G40159 and ICA Quimbaya) formed a group with less increase of root diameter (< 9.43%) and were significantly different ($P < 0.05$, REGWQ test) from two genotypes, G35066-1Q and VAX1 that were classified as sensitive (> 20.23 %).

Combination of fine and extensive roots: Genotypes were identified that combine both lower inhibition of tap root elongation and lower increase of root diameter based on data from the hydroponic experiment with or without Al-stress (Figure 2). Five genotypes from different bean species and gene pools (G35464-5Q, G35346-3Q, G40159, ICA Quimbaya and VAX6) were outstanding for minimizing inhibition of TRER and increase of root diameter, while another *P. coccineus* accession (G35346-2Q) presented inhibition of root elongation of 30.87% and was only slightly superior to the mean for increase of root diameter (13.1%). Al sensitive line VAX1 presented inhibition of TRER and increase of root diameter of 60.28% and 20.2%, respectively. The highest increase of root diameter by 20 µM Al was shown by G35066-1Q while root elongation of VAX3 was the most inhibited among all 11 genotypes.

Table 2a: Influence of Al-stress (with (20 µM Al) and without (0 µM Al)) on total root length (TRL), tap root elongation (TRE) at 48 h and at 5 days, root dry biomass weight (RDBW), mean root diameter (MRD), specific root length (SRL) and number of root tips (RTP) for 11 bean genotypes from three *Phaseolus* species grown in hydroponic system.

Genotypes	TRL (m)		TRE 48h (cm)		TRE 5d (cm)		RDBW (g)		MRD (mm)		SRL (m.g^{-1})		RTP (no.)	
	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al
G 35464-5Q	8.4	11.7	22.4	22.3	30.8	31.1	0.13	0.12	0.57	0.5	68.2	91.4	926.3	1924.2
G 35346-2Q	8.1	11.0	17.3	20.0	26.4	28.7	0.15	0.16	0.69	0.61	53.6	72.8	668.4	1307.4
G 35346-3Q	5.5	9.7	16.7	19.6	24.0	27.1	0.11	0.14	0.62	0.57	49.3	70.5	472.6	1395.0
G 35066-1Q	4.2	8.8	16.0	18.4	20.9	22.6	0.1	0.12	0.7	0.55	41.2	75.3	374.3	1325.6
ICA Quimbaya	3.2	5.9	17.4	19.2	20.2	24.2	0.07	0.09	0.63	0.54	44.3	62.6	364.9	1221.2
I.J.R.	2.8	4.7	16.4	18.6	18.9	23.9	0.06	0.09	0.6	0.55	53.7	61.6	396.8	965.3
VAX 1	2.5	6.4	16.4	21.0	17.0	26.2	0.04	0.06	0.57	0.45	59.8	114.6	284.0	1110.6
G 40159	2.4	4.0	16.6	20.5	17.8	23.5	0.03	0.04	0.47	0.42	86.1	111.4	306.9	707.0
SER 16	2.0	3.7	15.8	18.0	17.0	23.0	0.03	0.05	0.58	0.51	64.1	75.9	228.8	561.1
VAX 3	2.0	4.9	16.1	20.3	18.2	26.5	0.03	0.06	0.56	0.48	63.2	91.4	218.9	802.9
VAX 6	1.9	3.3	13.0	15.9	14.3	19.7	0.04	0.06	0.63	0.58	49.9	56.0	177.3	422.1
Mean	3.5	6.3	16.5	19.4	19.6	24.8	0.07	0.08	0.6	0.52	56.3	80.5	360.7	1028.8
$LSD_{0.05}$	0.3	0.4	0.6	0.7	0.8	0.8	0.01	0.02	0.03	0.02	15.3	25.7	4.8	7.0

Table 2b. Correlation coefficients and mean squares for total root length (TRL), tap root elongation (TRE) at 48 hour and 5days, root dry biomass weight (RDBW), mean root diameter (MRD), specific root length (SRL), and number of root tips (RTP) for 11 bean genotypes under hydroponics screening with two level of Al (20 µM Al and 0 µM Al).

Traits/ Source	Al level/DF	TRL	TRE48h	TRE5d	RDBW	MRD	SRL	RTP
TRL	0	1						
	20	1						
TRE48h	0	0.55 (ns)	1					
	20	0.77**	1					
TRE5d	0	0.78**	0.74**	1				
	20	0.92***	0.74**	1				
RDBW	0	0.85***	0.13 (ns)	0.58 (ns)	1			
	20	0.91***	0.59 (ns)	0.83**	1			
MRD	0	0.14 (ns)	-0.59 (ns)	-0.04 (ns)	0.61*	1		
	20	0.29 (ns)	-0.13 (ns)	0.24 (ns)	0.57 (ns)	1		
SRL	0	0.17 (ns)	0.72*	0.26 (ns)	-0.34 (ns)	-0.84***	1	
	20	-0.23 (ns)	0.07 (ns)	-0.20 (ns)	-0.46 (ns)	-0.87***	1	
RTP	0	0.95***	0.43 (ns)	0.66*	0.86***	0.16 (ns)	0.04 (ns)	1
	20	0.96***	0.78**	0.91***	0.84***	0.20 (ns)	-0.11 (ns)	1
Level of Al	1	12.34***	4.54*	10.93***	0.0062***	0.0494***	11894.35**	5158.2***
Rep. (Al level)	40	0.099 (ns)	0.46 (ns)	0.68 (ns)	0.0002 (ns)	0.0008 (ns)	518.5 (ns)	34.97 (ns)
Genotype	10	4.93***	0.86*	2.33***	0.0157***	0.0184***	5894.5***	525.5***
Gen. X Al level	10	2.23*	0.1 (ns)	0.45 (ns)	0.0001 (ns)	0.0015*	1713.6***	73.77**
Error	236	0.12	0.37	0.53	0.0002	0.0007	377.5	30.3

*, ** and *** Statistical significance at the 0.5, 0.01, and 0.001 probability levels, respectively; ns, non significant.

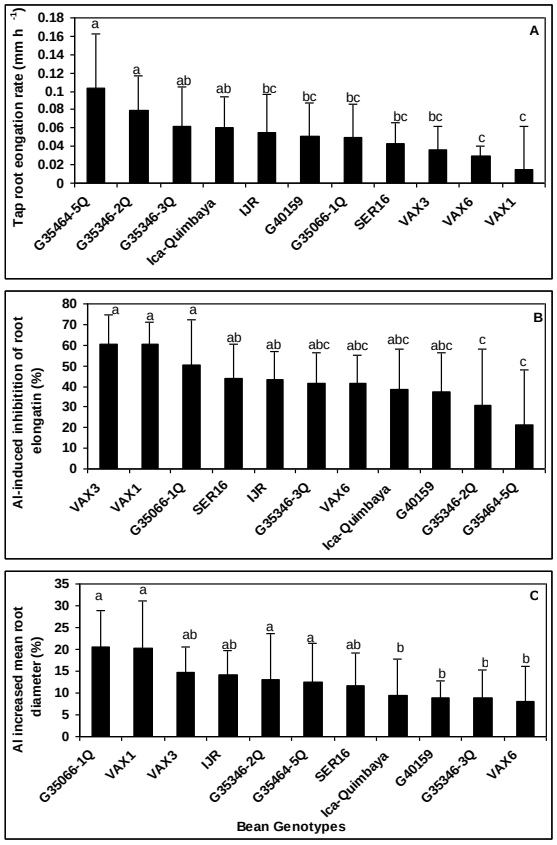

Figure 1. Tap root elongation rate (TRER) under high Al (20 µM Al) (A), and per cent Al-induced inhibition of tap root elongation rate (B) and increase of root mean diameter (C) of 11 bean genotypes under hydroponic screening with two levels of Al (20 µM Al and 0 µM Al) at pH 4.5. Bars represent means ± SD, with 4 replicates. Different letters indicate differences between at $P < 0.05$ (REDGRQ test).

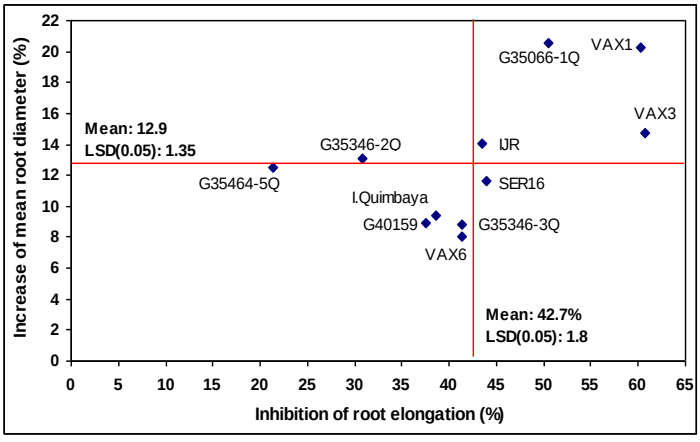

Figure 2. Relationship between Al induced inhibition of tap root elongation rate and increase of mean root diameter of 11 bean genotypes grown for 48 h in a nutrient solution containing 5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 at pH 4.5 under hydroponic screening with two levels of Al (20 µM Al and 0 µM Al).

Evaluation for Al-toxic Acid Soil Tolerance

Root development and distribution: The effects of Al level were highly significant ($P < 0.001$) for all root parameters except for mean root diameter (MRD) (Table 3b). Average values for low and high Al treatments respectively were: total root length (TRL), 59 and 27.4 m; mean root diameter (MRD), 0.30 and 0.29 mm; rooting depth (RDP), 67.1 and 56.7 cm; and specific root length (SRL), 106.0 and 78.7 $m.g^{-1}$. Genotypic differences were highly significant ($P < 0.001$) for TRL, MRD, SRL and RDP at 29 d. Acid soil tolerant genotypes G35346-2Q, G35346-3Q and G353464-5Q maintained good root structure under Al stress. Genotype x Al level interaction was significant for SRL (0.05) and RDP at 29 d (0.01). The genotype ranking based on variation of TRL, MRD, RDP and SRL of plants grown in soil tubes was different under Al treatment and control (Table 3a). In high Al saturation soil treatment, total root length was correlated ($P < 0.01$) with only two root traits, RDP at 29 d ($r = 0.78$) and R:S ratio ($r = 0.81$); whereas mean root diameter was highly correlated with RDP of 29 day-old plants ($r = 0.92$, $P < 0.001$) (Table 3b).

Table 3a: Influence of acid soil stress (high aluminum saturation, H-Al; low aluminum saturation, L-Al) on total root length (TRL), mean room diameter (MRD), specific root length (SRL) and rooting depth (RDP) for 29 days-old plants of 11 bean genotypes from three *Phaseolus* species grown in soil tubes under well watered conditions.

	TRL (m)		MRD (mm)		RDP (cm) at 29d		SRL ($m.g^{-1}$)	
Genotypes	**HAl**	**LAl**	**HAl**	**LAl**	**HAl**	**LAl**	**HAl**	**LAl**
G 35346-2Q	62.0	76.8	0.33	0.35	74.3	71.3	88.8	88.6
G 35346-3Q	40.1	73.0	0.35	0.37	75.0	75.0	84.8	79.3
G 35464-5Q	40.0	66.1	0.34	0.33	68.0	66.7	96.1	89.8
G 35066-1Q	24.6	47.1	0.30	0.29	57.0	64.3	65.8	111.8
VAX 1	23.0	56.1	0.24	0.24	40.8	62.7	61.1	126.2
I. Quimbaya	22.8	55.1	0.31	0.31	62.0	65.7	63.1	90.4
G 40159	21.3	55.5	0.25	0.27	53.0	69.0	134.3	135.7
VAX 6	19.1	50.0	0.28	0.27	55.0	57.0	71.6	106.5
I.J.R.	18.1	57.2	0.32	0.31	55.8	75.0	61.9	113.0
SER 16	15.4	53.1	0.26	0.28	37.7	66.3	71.3	109.5
VAX 3	15.0	59.5	0.26	0.27	45.0	64.7	67.1	114.9
Mean	27.4	59.0	0.29	0.30	56.7	67.1	78.7	106.0
$LSD_{0.05}$	22.6	32.0	0.09	0.07	24.2	17.02	45.7	35.1

Table 3b: Correlation coefficients and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), and root depth (RDP) of 29 days-old plants, leaf area (LA), shoot dry biomass weight (SDBW) and root:shoot ratio (R:S ratio) for 11 bean genotypes under high and low aluminum saturated soil in a soil tube evaluation.

Traits/Source	**Al level/DF**	**TRL**	**MRD**	**SRL**	**RDP29d**	**LA**	**SDBW**	**R:S ratio**
TRL	LAl	1						
	HAl	1						
MRD	LAl	0.53 (ns)	1					
	HAl	0.58 (ns)	1					
SRL	LAl	-0.39 (ns)	-0.79**	1				
	HAl	0.33 (ns)	0.27 (ns)	1				
RDP29d	LAl	0.65*	0.77**	-0.33 (ns)	1			
	HAl	0.78**	0.92***	0.34 (ns)	1			
LA	LAl	0.15 (ns)	0.35 (ns)	0.02 (ns)	0.64*	1		

Continued Table 3b.....

Table 3b: Correlation coefficients and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), and root depth (RDP) of 29 days-old plants, leaf area (LA), shoot dry biomass weight (SDBW) and root:shoot ratio (R:S ratio) for 11 bean genotypes under high and low aluminum saturated soil in a soil tube evaluation.

Traits/Source	Al level/DF	TRL	MRD	SRL	RDP29d	LA	SDBW	R:S ratio
	HAl	0.52 (ns)	0.14 (ns)	0.22 (ns)	0.31 (ns)	1		
SDBW	LAl	0.64*	0.57 (ns)	-0.26 (ns)	0.82**	0.69*	1	
	HAl	0.50 (ns)	0.38 (ns)	0.46 (ns)	0.47 (ns)	0.81**	1	
R:S ratio	LAl	0.47 (ns)	0.68*	-0.79**	0.24 (ns)	-0.29 (ns)	-0.02 (ns)	1
	HAl	0.81**	0.70*	0.05 (ns)	0.71*	0.15 (ns)	0.11 (ns)	1
Level of Al	1	16524.7***	0.0007 (ns)	12236.7***	1774.25***	811056.2***	19.58***	0.34***
Rep. (Al level)	4	28.77 (ns)	0.0009 (ns)	299.3 (ns)	420.9 (ns)	2991.6 (ns)	0.19 (ns)	0.008 (ns)
Genotype	10	776.2***	0.009***	1342.06***	3747.87***	3235.02 (ns)	0.28*	0.06***
Gen. x Al level	10	100.38 (ns)	0.0003 (ns)	986.2**	1815.5$^{\mu}$	8193.2*	0.18 (ns)	0.012*
Error	40	138.97	0.0012	301.16	79.23	3773.3	0.119	0.004

*, ** and *** Statistical significance at the 0.5, 0.01, and 0.001 probability levels, respectively; ns, non significant.

Evaluation for Tolerance to Combined Stress of Al-toxic Acid Soil and Drought

Combined stress of Al-toxic acid soil and drought: Treatment effects of Al levels x water regimes were highly significant ($P < 0.001$) for TRL and SRL, and significant ($P < 0.05$) for MRD and RDP. Combined stress of Al-toxic soil and drought was the most inhibitory to TRL, followed by drought alone, and then by Al alone, based on the treatment averages. However, as expected, Al-stress alone was more inhibitory than drought to specific root length (SRL) and rooting depth (RDP). Genotypes were significantly different ($P < 0.001$) for all root traits considered (TRL, MRD SRL, and RDP) (Table 4b). Two sister lines of *P. coccineus*, G35346-3Q and G35346-2Q, were the most tolerant to combined stress, presenting the highest values of TRL and maintaining a deeper root system (Table 4a). They were also the two best under Al-stress alone but showed difficulties to develop a deep rooting system under water stress alone. Other relatively tolerant genotypes in combined stress were G35066-1Q and ICA Quimbaya. Interactions of genotype x treatment (Al and water stress) were significant for total root length and specific root length ($P < 0.01$), and highly significant for root depth ($P < 0.001$) (Table 4b). Combined stress of aluminum and drought is generally more damaging than each single stress considered separately. However, in this study an unexpected interaction between these two abiotic stresses was observed. *P. coccineus* accessions (G35346-3Q, G35346-2Q, and G35066-1Q) and to lesser extent ICA Quimbaya developed more roots, and showed deeper rooting with the combined Al and drought stress than with drought stress alone. Total root length was correlated with mean root diameter ($r = 0.43$, $P < 0.05$), and with root depth ($r = 0.79$, $P < 0.001$) (Table 4b). MRD was negatively correlated with SRL ($r = -0.62$, $P < 0.001$).

Total root length per plant across experiments: Total root length was analysed at two levels of Al treatment both in hydroponic and soil tube experiments. There was a strong linear relationship between total root length per plant under 20 µM Al and the control with 0 µM Al in hydroponic system ($R^2 = 0.89$) (Figure 3a). For soil tube system, the linear relationship between total root length under low Al and high Al saturation was also strong ($R^2 = 0.71$) (Figure 3b). Four *P. coccineus* accessions (G35464-5Q, G35346-2Q, G35346-3Q, and G35066-1Q) showed greater values of TRL both with and without Al in hydroponic system. Similar observations were made for soil tube studies where three *P. coccineus* (G35346-2Q, G35346-3Q, and G35464-5Q) accessions maintained good root development in soil with either low or high Al saturation.

Table 4a: Influence of individual and combined stress factors of acid soil (H-Al, high aluminum; L-Al, low aluminum) and drought (WW, well watered; WS, water stress) on total root length (TRL), mean root diameter (MRD), Specific root length (SRL) and rooting depth (RDP) for 33 days-old plants of 11 bean genotypes from three *Phaseolus* species grown in soil tubes.

Genotypes	TRL (m)				MRD (mm)				RDP at 33d (cm)				SRL (m.g^{-1})			
	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS
G 35346 3Q	73.7	30.4	38.7	42.5	0.38	0.35	0.42	0.39	72.2	56.6	66.8	75.0	68.6	67.6	56.6	57.4
G 35346 2Q	56.9	28.6	66.5	37.7	0.36	0.36	0.37	0.37	62.9	59.0	68.0	73.0	76.7	63.4	70.6	61.7
G 35066 1Q	25.6	18.8	31.7	27.8	0.35	0.34	0.38	0.38	45.8	59.0	67.8	62.0	58.7	51.9	54.5	58.6
I. Quimbaya	32.4	17.2	29.2	22.5	0.36	0.41	0.39	0.39	68.1	65.5	66.0	69.0	66.3	54.9	57.7	53.7
I.J.R.	44.0	28.4	22.7	21.9	0.38	0.38	0.41	0.4	75.0	75.0	72.0	71.0	64.9	56.6	52.6	47.1
G 35464 5Q	42.3	26.7	35.7	19.9	0.35	0.37	0.43	0.4	60.5	61.4	69.2	59.8	66.3	59.3	47.9	43.5
VAX 1	34.0	25.4	25.4	16.4	0.33	0.33	0.35	0.34	58.5	71.6	59.5	65.7	82.6	75.9	73.2	64.1
G 40159	54.1	22.9	20.5	15.1	0.32	0.34	0.33	0.33	75.0	71.5	64.5	57.0	101.9	83.5	86.9	84.1
VAX 3	42.7	19.7	15.3	12.2	0.36	0.34	0.34	0.35	67.7	68.2	46.9	49.0	83.1	75.4	56.9	59.0
SER 16	58.9	21.9	14.6	11.9	0.36	0.34	0.35	0.37	70.3	72.5	60.0	48.3	80.4	72.4	59.7	59.0
VAX 6	29.9	16.7	12.1	11.8	0.35	0.35	0.3	0.41	68.2	66.7	44.2	42.9	68.8	69.4	59.2	59.6
Mean	45.0	23.3	28.4	21.8	0.35	0.36	0.37	0.37	65.8	66.1	62.3	61.2	74.4	66.4	61.4	58.9
$LSD_{0.05}$	45.7	11.4	14.0	9.4	0.07	0.05	0.07	0.08	22.6	10.0	14.9	18.1	13.0	15.7	10.6	12.7

Table 4b: Correlation coefficient and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), and root depth at 33 days-old plants (RDP at 33d), leaf area (LA), shoot dry biomass weight (SDBW), root:shoot ratio for 11 bean genotypes (23 days under drought) under screening for combined stress of Al and drought.

Traits/Source	df	TRL	MRD	RDP 23d	SRL	LA	SBDW	R:S ratio
TRL	_	1						
MRD	_	0.43*	1					
RDP at 23d	_	0.79***	0.27 (ns)	1				
SRL	_	-0.2 (ns)	-0.62***	-0.11 (ns)	1			
LA	_	0.62***	0.56***	0.58***	-0.39*	1		
SDBW	_	0.82***	0.55***	0.59***	-0.42*	0.62***	1	
R:S ratio	_	0.57***	0.19 (ns)	0.59***	-0.06 (ns)	0.32 (ns)	0.10 (ns)	1
Al & Water reg.	3	3716.93***	0.003*	206.45*	1533.36***	331.127***	8.629***	0.5226***
Rep. (Al & Wr)	8	167254 (ns)	0.001 (ns)	61.58 (ns)	115.38**	2312.9 (ns)	0.123 (ns)	0.05 (ns)
Gen.	10	1023.53***	0.005***	313.86***	1244.17***	13.549**	0.435*	0,355***
Gen. x (Al & Wr)	30	240.33**	0.001 (ns)	208.53***	65.93**	17.211***	0.432**	0,355***
Error	80	113.45	0.0008	52.59	31.4	5234.6	0.22	0.032

*, ** and *** Statistical significance at the 0.5, 0.01, and 0.001 probability levels, respectively; ns, non significant.

The two screening methods were highly correlated ($r = 0.85$, $P < 0.001$) for TRL in soil with high Al saturation and nutrient solution with 20 µM Al (Figure 3c). A significant but lower correlation ($r = 0.62$, $P < 0.05$) was found between TRL in low Al saturation soil and nutrient solution without Al in hydroponic system (Figure 3d). Three *P. coccineus* accessions (G35346-2Q, G35346-3Q, and G35464-5Q) were found to be outstanding in their level of Al resistance under both hydroponic and soil tube systems.

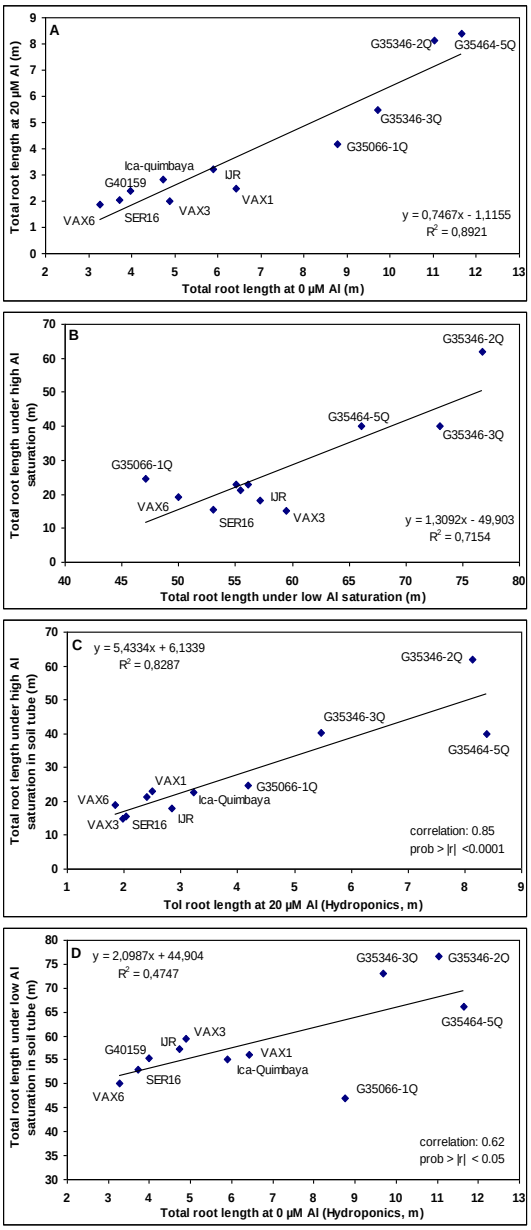

Figure 3. Relationship between total root length of 11 bean genotypes in Al treatment in hydroponics and soil tube evaluation, and controls for the two screening methods. The Al treatment was characterized by a soil with 76% Al soil saturation (pH = 4.1) for top-soil of the cylinder (0-10 cm), and 83% saturation for subsoil (10-75 cm) (pH = 4.14). The low Al saturation was made by tubes packed for topsoil with soil (28 % Al saturation, pH = 4.45) and for subsoil (58 % Al saturation, pH = 4.29). Hydroponic evaluation was done in a nutrient solution containing 20 µM Al (with 0 µM Al for the control). The roots of seedlings were harvested after 5 days of Al treatment.

Root and shoot attributes across experiments: Genotype ranking based on shoot vigor was made based on leaf area (LA) and shoot dry biomass weight (SDBW) under individual and combined stress of Al and drought. G35346-3Q was the best genotype for these two shoot traits for combined stress but was intermediate for Al alone for both traits, and was very poor for drought stress alone (Table 5). Genotype x Al level interaction was significant for LA and R:S ratio ($P < 0.05$) and a highly significant interaction was observed for genotype x (Al and water regime) for LA and R:S ratio ($P < 0.001$), and SBDW ($P < 0.01$) (Table 4b).

The relationships between shoot traits (LA and SDBW) and TRL in Al stress alone and in combined stress of Al and drought in soil tube system were analysed. Linear regression of LA on TRL in Al stress for the 11 genotypes showed positive relationship (R^2 = 0.65; P < 0.05) (Figure 4a), and a similar relationship was observed between SDBW and TRL (R^2 = 0.69) (Figure 4b) in Al stress. Relationship between SDBW and TRL in combined stress of Al and drought also showed a strong positive relationship (R^2 = 0.66) (Figure 4c). All *P. vulgaris* cultivars and the *P. acutifolius* accession performed poorly in root development and LA production with the exception of an Andean bean type Indeterminate Jamaica Red (IJR) that was intermediate (Figure 4a). G35346-2Q was the best genotype under Al-stress alone for combining an extensive root system, large leaves and vigorous seedlings, while G35346-3Q was found to be the best with similar potential under combined stress of Al and drought.

Discussion

Different screening methods for response to Al produce different sorts of data which in turn can generate derived parameters. These can serve as selection criteria for breeding programs either individually or in combination. A critical analysis of these parameters should lead to identification of the most relevant to employ for selection. In the current study, the contribution of specific shoot traits (biomass accumulation; leaf area production) is considered to be an important indicator of the potential to eventually contribute to seed yield. We will first discuss the results in the hydroponic screening, and then look at their relationship to the soil tube method and especially shoot parameters.

Al Resistance in Hydroponic System

Total root length and tap root elongation: Phenotypic characterization of 11 bean genotypes for Al resistance in hydroponic system revealed that all genotypes were affected by Al toxicity (Table 2a). Root vigor as reflected in root system size (TRL) and tap root elongation rate (TRER) of Al-stressed plants compared to non-stressed plants revealed changes in root growth and function, and were interpreted as indicators of Al resistance (Manrique et al., 2006). In contrast López-Marín et al. (2009) found that while bean genotype G19833 showed a higher tap root elongation rate than DOR364 in both control and Al treatment solutions, differences in TRL between the two genotypes were less notable. In wheat also, clear differences in root length was observed between Al-tolerant and Al-sensitive wheat seedlings at 20 µM Al, with 50% inhibition of root growth (Delhaize et al., 1993). Our results indicated that 20 µM Al significantly affected root system of sensitive bean genotypes compared to Al resistant *P. coccineus* accessions. Genetic differences under Al-toxic stress were associated with TRL, TRE 5d, RDBW, MRD, SRL and RTP (Table 2b). Similar results were found by López-Marín et al. (2009) for Genotype and Genotype x Al treatment interaction. In contrast, in our study Genotype x Al treatment was significant only at P < 0.05 for TRL and MRD and highly significant for RTP (P < 0.01) and SRL (P < 0.001). No significant interactions of Genotype x Al treatment were observed in hydroponics for TRE indicating that the 11 genotypes did not show differential response to Al stress for this trait. Positive and high correlations suggested a strong relationship between RTP, TRL, and TRE at 5 d for evaluation of beans in the hydroponic system.

Root tips and branching: Lower Al contents were observed in root tips of Al-resistant common bean compared with Al-sensitive common bean after 1 d or 3 d of Al treatment, respectively (Mugai et al., 2000; Shen et al., 2002; and Rangel et al., 2007). This could explain strong inhibition of TRER observed in sensitive genotypes. In a study of Al-induced inhibition of root development, Villagarcia et al. (2001) reported that number of basal roots and branching from tap root were clearly reduced in all soybean genotypes. We found similar results for number of root tips (root branching) and for SRL which is affected by number of fine branches. As expected, SRL exhibited a strong negative correlation with average root diameter. However, ranking the 11 bean genotypes by SRL values as a trait for determining Al-resistance did not agree with ranking of any other root characteristics (Table 2a). Thus SRL alone may not serve as a useful trait to evaluate Al resistance in common bean.

Table 5: Leaf area (LA), shoot dry biomass weight (SDBW) and root-shoot ratio (R:S) under Al soil tube experiment and individual and combined stress of Al and drought experiment for 11 bean genotypes including 4 *P. coccineus* accessions, 1 *P. acutifolius* and 6 *P. vulgaris* under soil tube greenhouse screening

	Individual stress of Al						Individual and combined stress of Al and drought											
	LA (cm^2)		SDBW (g)		R:S ratio		LA (cm^2)				SDBW (g)				R:S ratio			
Genotypes	HAl	LAl	HAl	LAl	HAl	LAl	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS
G 35346-3Q	66.6	399.1	0.67	2.25	0.71	0.42	356.6	78.0	132.0	136.6	1.69	0.52	0.96	0.92	0.75	0.99	0.72	0.8
G 35346-2Q	212.2	300.3	1.40	1.90	0.52	0.48	317.4	93.2	152.8	106.8	1.66	0.72	0.86	0.6	0.45	0.75	1.08	1.03
I.J.R.	67.8	375.6	0.68	2.22	0.45	0.25	365.2	91.1	108.4	95.1	1.97	1.17	0.82	0.56	0.34	0.43	0.53	0.85
G 35464-5Q	136.4	304.9	1.13	2.10	0.56	0.35	164.5	123.1	189.8	91.0	1.21	0.91	1.19	0.77	0.49	0.51	0.63	0.62
G 35066-1Q	110.8	330.2	0.70	1.54	0.48	0.28	125.9	153.6	137.5	83.8	0.96	0.36	0.88	0.75	0.44	1.18	0.65	0.66
VAX 1	87.0	290.4	0.55	1.37	0.49	0.29	169.9	150.7	127.1	69.8	0.98	0.77	0.63	0.35	0.45	0.46	0.55	0.73
SER 16	73.1	341.7	0.73	1.86	0.33	0.25	564.1	157.4	60.9	62.3	3.13	0.98	0.6	0.36	0.24	0.32	0.4	0.58
I. Quimbaya	123.5	245.3	0.83	1.58	0.43	0.37	178.3	36.3	98.9	56.6	1.24	0.52	0.87	0.59	0.4	0.61	0.57	0.74
G 40159	92.2	333.4	0.82	2.07	0.19	0.19	418.7	136.7	109.5	43.1	2.49	1.14	0.69	0.44	0.2	0.25	0.34	0.42
VAX 6	61.5	291.7	0.55	1.76	0.44	0.25	260.5	105.1	55.2	40.2	1.57	0.68	0.62	0.32	0.29	0.36	0.34	0.62
VAX 3	69.7	326.8	0.50	1.91	0.37	0.24	350.2	134.9	70.9	29.6	1.89	0.67	1.09	0.32	0.3	0.39	0.5	0.68
Mean	100.1	321.8	0.78	1.87	0.45	0.31	297.4	114.6	113.0	74.1	1.71	0.77	0.84	0.54	0.4	0.57	0.57	0.7
$LSD_{0.05}$	148.6	140.0	0.75	0.87	0.18	0.12	318.7	84.1	56.6	61.6	1.9	0.57	0.92	0.25	0.3	0.6	0.29	0.37

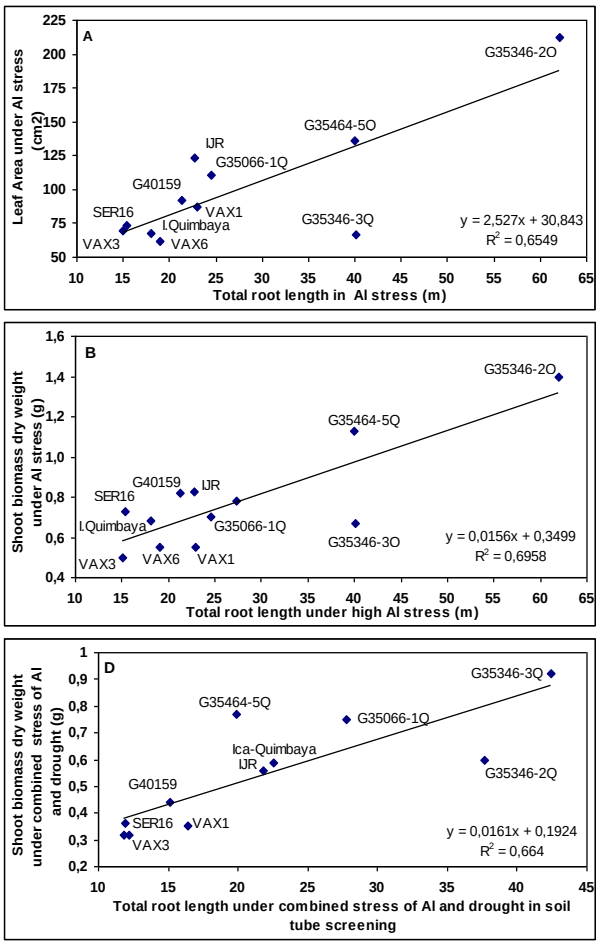

Figure 4. Relationship between total root length and shoot attributes of 11 bean genotypes under individual and combined stress of Al and drought. The Al treatment was characterized by a soil with 76% Al soil saturation and pH of 4.1 for top-soil of the cylinder (0-10 cm), and 83% saturation for subsoil (10-75 cm) with pH 4.14. The low Al saturation was made by tubes packed for topsoil with soil (28 % Al saturation, pH 4.45) and for subsoil (58 % Al saturation, pH 4.29). 29 days-old plants were harvested for the Al stress experiment. Water stress was imposed to plant after 10 days of initial growth for the third experiment (Individual and combined stress of Al and drought) and 23 days-old plants were harvested.

Tap root elongation rate: Phenotypic characterization of resistance to Al-toxicity stress based on TRER, inhibition of root elongation rate and increase of mean root diameter revealed differences in root growth per treatment period and in Al-induced changes on root morphology (Figure 1). Clarkson (1965) considered that a reduction in root growth rate was the most obvious consequence of Al treatment. The highest TRER in this study was found in three Al resistant *P. coccineus* accessions (G35464-5Q, G35346-2Q and G35346-3Q) and ICA Quimbaya compared to other genotypes, confirming the variability revealed previously between the two bean species observed before using hydroponic system (CIAT, 2005), and the level of Al resistance reported in ICA Quimbaya (Rangel et al., 2007). Growth rates of roots adapting to Al treatment are initially faster than normal suggesting that the early phases of recovery may involve growth stimulation (Bennet and Breen, 1991). Comparing ICA Quimbaya to Al-sensitive genotype VAX1 in hydroponics, Rangel et al. (2007) found that Al content reached higher levels in 0-5 mm root tip in Quimbaya up to 16 h of exposure to Al and it decreased by 24 h and that the higher levels of Al during the initial 16 h of exposure in Quimbaya did not lead to more severe inhibition of root elongation. We found similar results where the highest TRER (5 days) was associated with lesser inhibition of TRER in G35464-5Q and G35346-2Q. Kopittke et al. (2004) indicated that the higher growth

rate of mungbean (*Vigna radiata* L.) roots between days 3 to 6 resulted in the accumulation of detectable quantities of Al_{13} during the exposure time.

Inhibition of tap root elongation rate: The major Al toxicity symptom observed in plants is inhibition of root growth calculated from the comparison of root growth with and without toxic Al (Delhaize and Ryan, 1995; Marschner, 1991; Rhue and Grogan, 1977; Ryan et al., 1993). Root growth inhibition was detected within 2-4 days after the initiation of seed germination (Bennet et al., 1991). Significant genotypic differences in Al resistance in common bean were reported based on Al-inhibited root elongation in nutrient solution (Massot et *al*., 1999; Rangel et al., 2005; Manrique et al., 2006). Our results showed that at 48 h there was no difference for relative tap root elongation among *P. coccineus* accessions and we therefore extended the Al exposure time to 5 days to detect genotypic variability. Using the same method with two common bean genotypes (ICA Quimbaya and VAX1), Rangel et al. (2007) showed that root elongation was greatly inhibited at 36 h. Genotypic ranking for inhibition of TRER in the current study indicated that G35464-5Q and G35346-2Q were the most Al resistant with less inhibition (Figure1b). G35346-3Q and ICA Quimbaya were found to be intermediate in their level of Al resistance based on Al-induced inhibition of root growth but were among the best in developing roots with a low increase of mean diameter (Figure 1c). Fine roots are believed to be more important than thick roots in nutrient and water absorption, and therefore more important in terms of Al resistance (Eisenstat, 1992; Villagarcia et al., 2001). Two contrasting parents used by López-Marín et al. (2009) to generate recombinant inbred lines (RIL) presented mean root diameter significantly different, DOR364 with finer roots than G19833. According to Liu et al. (2010) in a study on pulse crops, classification of fine roots needs to be further refined. In our work we found that the increase of average root diameter was associated with root growth inhibition ($r = 0.33$, $P < 0.001$; data not reported). Similar results were found before in evaluation of Al resistance among 52 genotypes of common bean (CIAT, 2005).

Al-resistance and Acid Soil Tolerance

Relationship of root traits in hydroponic and soil tube experiments: Several authors have examined the relationship in response between screening methods. Narasimhmoorthy et al. (2007) compared three methods including hydroponics, soil, and root staining for evaluation of Al tolerance in *Medicago truncatula* (Barrel Meic) germplasm and found a weak correlation, suggesting that each technique is distinct and cannot be substituted for each other. A large discrepancy between hydroponics-based ratings of seedlings and sand-culture-based ratings of soybean plants was found when Al tolerance was expressed as percentage of controls, and correlations between sand culture and hydroponics-based results were found to be low (Villagarcia et al., 2001). Horst and Klotz (1990) compared 31 soybean genotypes using hydroponics and soil systems and detected a positive though non-significant relationship ($r = 0.79$). In contrast, Campbell and Carter (1990) demonstrated in their experiments a good agreement between Al tolerance ratings determined in solution culture and pots with soil in the greenhouse when expressed as percentage of the control (PC) treatment. Recent research on peanut indicated that root characteristics of plants grown in hydroponics were closely related with those of plants grown with soil in small pot conditions (Girdthai et al., 2010). Our results from the hydroponic system showed that differences in Al resistance among the eleven bean genotypes to high Al in solution was associated with four root traits, TRL, SRL, number of root tips and MRD (Table 4b); whereas tolerance to Al-toxic acid soil was associated with two root traits, SRL and RDP (Table 3b). When we compared the results from the hydroponic system with soil based evaluation of these bean genotypes we found a strong relationship between Al resistance and acid soil tolerance for some specific root traits. Significant correlations were found between three root traits from hydroponic evaluation (TRL, TRE 5 d and SRL) and three other root traits from soil tube evaluation (TRL, RDP at 29 d and MRD). No significant correlation was found between SRL in hydroponic and soil tube experiments indicating little or no relation between the two methods for this variable. Genotypic ranking based on SRL in nutrient solution (with 20 µM Al) did not agree with the ranking in Al-toxic acid soil. Hydroponic evaluation identified the soybean cultivar Perry as Al sensitive even though it had been found to be tolerant in soil-based assays with older plants (Armiger et al., 1968; Devine et al., 1979; Sapra et al., 1982; Horst and Klotz, 1990; Foy et al., 1969 and 1992). VAX1 which was found to be Al sensitive in hydroponic system was found to be acid soil tolerant under field conditions because of its abundant adventitious root system that helps to avoid Al toxicity. To avoid misleading conclusions it is better to consider the two methods separately. The hydroponic system is useful to assess the level of Al resistance whereas the soil tube system is suitable to evaluate tolerance to Al-toxic acid soil conditions.

Complementarity Between the Two Screening Methods

Villagarcia et al. (2001) found that despite the imposition of stress to approximately the same degree in hydroponic and sand culture systems, the genotypic variation in Al tolerance of soybean was much greater in hydroponics as evidenced by the following: much greater Al x genotype interaction, increased genotypic variation in response to stress, a much wider range in Al tolerance expressed as per cent control (PC), a lower correlation between genotype means for Al-free and Al-stress treatments, and a greater correlation between ratings under Al-stress conditions and PC. We did not find exactly the same genotype ranking between hydroponic evaluation for Al resistance and soil based evaluation for acid soil tolerance revealing their complimentarity. Furthermore, each screening method demonstrated some particularity. For example, the hydroponic system enabled quantification of number of root tips (the most Al-sensitive part of the root) that was not possible with soil tubes after cutting the tubes at different soil depths, whereas soil based screening revealed rooting ability to penetrate Al-toxic soil. Narasimhamoorthy et al. (2007) concluded that a combination of soil-based screening and hydroponics could be essential to identify Al tolerant genotypes possessing multiple Al tolerance mechanisms. Our results are consistent with this report.

Response of shoot traits

The ability of roots to sense and respond to stress largely determines how successful they can be in adaptation to a changing soil environment (Wolters and Jürgens, 2009). Acid soils with high levels of Al impede root growth, causing increased crop sensitivity to drought and decreased nutrient acquisition (Bianchi-Hall et al., 2000). Shoot traits ought to reflect the effectiveness of the root system in obtaining water and nutrients, and should therefore respond to Al resistance in the roots. Compared to the control with low Al saturation and well watered soil, the means of shoot attributes such as LA, SDBW and R:S ratio showed not only the effects of the stress but also genetic variability under Al alone, drought alone and combined stress of Al and drought (Table 5). Villagarcia et al. (2001) found that shoot weight under Al-stress conditions in 18 day-old plants was significantly correlated to seedling ratings of Al tolerance (r = 70*), while root weight and Relative root surface area (RRSA) were associated to a lesser degree (r = 0.50 and 0.45, respectively). Our results confirmed this assumption insomuch as extensive leaf area was accompanied by strong shoot biomass investment in stems, branches and petioles. The genotypes G35346-2Q, G35464-5Q and ICA Quimbaya were outstanding under Al stress expressing a deeper root system (Table 3a) and maintaining a certain acceptable level of shoot development when sensitive genotypes were highly affected (Table 5). On the other hand, Al sensitive genotypes SER16, G40159 and VAX1 showed better shoot growth under drought stress than Al resistant genotypes did (Table 5).

Roots can exert indirect control on leaf growth which depends on the supply of cytokinins and water from roots (Lambers et al., 1995). High root length is an important characteristic for the acquisition of nutrients at low availability (Ryser and Lambers, 1995). Identification of a bean genotype that combines mechanisms for better biomass partitioning and an extensive root system to explore the soil volume more effectively will be an important achievement for this study. At low nutrient availability in short-term experiments some species with a high potential root growth rate (RGR) still grow faster than those with low potential RGR and have greater capacity to acquire nutrients (Chapin, 1980; Lambers and Poorter, 1992; Ryser and Lambers, 1995). Individual stress of Al on 29 day-old plants in soil tube experiment in this study revealed strong relationships between TRL and leaf area, $R^2 = 0.65$ (Figure 4a); and between TRL and SDBW, $R^2 = 0.69$ (Figure 4b). G35346-3Q favoured root growth at the expense of shoot growth while G35464-5Q was intermediate. G35346-2Q showed a pattern of high biomass allocation in leaves stems and roots; confirming this ability for Al resistance in beans. Combined stress of Al and drought (Figure 4c) showed also a high relationship between shoot biomass dry weight and total root length ($R^2 = 0.66$). G35346-3Q was superior in biomass accumulation in the whole plant and is recommended here as a source of Al resistance when the target environment is under combined stress conditions.

Tolerance to Individual Versus Combined Stress of Al-toxic Soil and Drought

When the 11 bean genotypes experienced water deficit in addition to Al-toxicity stress we found that physiological parameters varied from those in either unstressed conditions or under individual stress factors. In this study differences in resistance to combined stress of Al and drought were associated with three root traits, RDP at 33 d ($P < 0.001$), TRL ($P < 0.05$) and SRL ($P < 0.05$) (Table 4b). In response to individual and combined stress of Al and drought some genotypes such G35346-2Q and G35346-3Q maintained high R:S ratio whereby Mesoamerican bean genotypes SER16 and VAX6 showed low R:S ratio (Table 5). Rankings of genotypes under Al stress alone versus combined stress of Al and drought were different, suggesting that Al-resistant lines are not necessarily tolerant to combined stress. In Al-

toxic soil, TRL of 33 day-old plants correlated with R:S ratio (r = 0.57**). The effect of water stress combined with Al-toxicity in soil led to higher average reduction of TRL, LA, and biomass accumulation. *Phaseolus vulgaris* genotypes that expressed high values of LA and shoot biomass under drought stress, but were strongly affected by Al-toxic acid soil, were more sensitive in shoot parameters under combined stress factors of Al and drought (Table 5a), indicating that their capacity to acquire nutrients and water for shoot growth was reduced. Samac and Tesfaye (2003) found that root tips affected by Al were stubby due to inhibition of cell elongation and cell division, and concluded that the restricted root system impaired nutrient and water uptake making the plant more susceptible to drought stress.

A combination of environmental stresses can alter plant metabolism in a novel manner that may be different from that caused by each of the different stresses applied individually, and require a new type of response that would not have been induced by each of the individual stresses (Rizhsky et al., 2002). When we compared combined stress of Al and drought to each of these stress alone we found an unusual response with *P. coccineus* (G35346-3Q, G35346-2Q, G35066-1Q) and to lesser extent ICA Quimbaya. These presented more and deeper roots under combined stress than with drought alone (Table 4a). Aluminum was able to ameliorate the effects of drought in these genotypes, while in others the added stress of Al on top of drought was deleterious as expected. Recent work showed that in the presence of Al stress, PEG 6000 (polyethylene glycol)-induced osmotic (drought) stress lead to amelioration of Al-induced inhibition of root elongation in the Al-sensitive genotype VAX 1 (Yang et al., 2010). The osmotic stress-inhibited Al accumulation in root apices and thus reduced Al-induced inhibition of root elongation in VAX 1 was shown to be related to the alteration of cell wall porosity resulting from osmotic stress-induced dehydration of the root apoplast.

Identification of Aluminum and Drought Resistant Bean Genotypes

There is considerable variability in Al tolerance within some species and this has been useful to breeders in developing Al-tolerant cultivars of various crops (Delhaize and Ryan, 1995). Comparing TRL between the two treatments (with and without Al) used in hydroponic evaluation for Al resistance (Figure 3a) we found that the relationship between them was high (R^2 = 0.89) with a highly significant correlation (P < 0.001). Genotype x Al level interaction was also significant but at P <0.05. Effects of both aluminum and genotype were highly significant. In contrast genotype x Al treatment was not significant in soil tube system suggesting that whatever effect of Al that resulted in much less TRL was not an effect for which lines express differential resistance for Al. The relationship of TRL with and without Al stress for soil-based screening (Figure 3b) was also strong (R^2 = 0.71), and three resistant *P. coccineus* accessions form a separate group from all the other genotypes. The high relationship of stressed and unstressed treatments implies that differences among genotypes were constitutive. Urrea-Gomez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of Al-tolerant cultivars. Comparing the relationship between the two methods of Al resistance screening (Figure 3c) we found that the relation was most consistent between TRL values for the two systems of evaluation for Al resistance. Correlation between Al-toxic soil system and hydroponic system with 20 µM Al in nutrient solution was highly significant (r = 0.85; P < 0.001) whereas the correlation of the two controls (Figure 3d) was lower but still significant (r = 0.62, P < 0.05). These correlations were driven in large part by the presence of the *P. coccineus* accessions that performed well under both systems and conditions. Villagarcia et al. (2001) found that hydroponics-based assay of Al tolerance with seedlings and the sand-media nutrient-solution-based assays with somewhat older plants may both have a role in breeding. They concluded that some genetic sources will lend themselves well to hydroponics-based screening while others may not (Villagarcia et al., 2001). Using a hydroponic system, Narasimhamoorthy et al. (2007) concluded that genotypic vigor was an important factor to consider while applying selection for Al tolerance. Our strategy was to correlate data from hydroponic evaluation with Al-toxic soil evaluation to select best parental sources of Al resistance. We had identified two accessions of *P. coccineus*, G35346-2Q and G35464-5Q, as superior in Al resistance among the 11 genotypes tested. This finding constitutes a confirmation of previous results (CIAT, 2005) in which *Phaseolus coccineus* L. was found to be more Al resistant than *Phaseolus vulgaris* L. There is considerable genetic variability in Al and drought resistance within the two bean species and this will be useful to breeders in developing tolerant cultivars for these plant stresses individually or combined.

Conclusions

In this study, root phenotyping for Al resistance indicated that total root length (TRL) and tap root elongation rate (TRER) could be considered as the most important root characteristics when identifying Al resistant bean genotypes using hydroponics, while total root length (TRL) and rooting depth (RDP) are

the most useful root traits to be considered for evaluating tolerance to Al-toxic acid soil. These root traits had shown the sensitivity of genotypes to toxic-Al stress through root growth inhibition. They predicted how roots of resistant genotypes continue to branch under Al stress and maintained their exploratory capacity in soil, and contributed to remobilization of photosynthates to the reproductive plant parts (L. Butare, S. Beebe and I. Rao, unpublished results).

The results from hydroponics and soil tube studies indicated that these two methods of evaluation were effective in screening for resistance to individual and combined stress factors of Al and drought. The greater level of Al-resistance found in *P. coccineus* genotypes (G35346-2Q and G35464-5Q) offers the opportunity to obtain much better resistance in common bean through interspecific crosses. Another *P. coccineus* accession G35346-3Q identified in this study showed ability to tolerate combined stress factors of Al and drought. The use of this genotype in common bean improvement for resistance to these two stress factors is likely to be more productive than considering resistance to them in isolation. Populations created from multiple stress resistance donors could be more stable and capable to produce grain under stress in the face of climate change.

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References

Armiger, W.H., C.D. Foy, A.L. Fleming, and B.E. Caldwell. 1968. Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminum. Agron. J. 60:67-70.

Bennet, R.J., and C.M. Breen. 1991. The aluminum signal: new dimensions to mechanisms of aluminum tolerance. Plant Soil 134:153-166.

Bianchi-Hall, C.M., T.E. Carter, Jr., T.W. Rufty, C. Arellano, H.R. Boerma, D.A. Ashley, and J.W. Burton. 1998. Heritability and resource allocation of aluminum tolerance derived from soybean PI 416937. Crop Sci. 38:513-522.

Bianchi-Hall, C.M., T.E. Carter, Jr., M.A. Bailey, M.A.R. Mian, T.W. Rufty, D.A. Ashley, H.R. Boerma, C. Arellano, R.S. Hussey, and W.A. Parrott. 2000. Aluminum tolerance associated with quantitative trait loci derived from soybean PI416937 in hydroponics. Crop Sci. 40:538-545.

Campbell, K.A.G., and T.E. Carter, Jr. 1990. Aluminum tolerance in soybean: I. Genotypic correlation and repeatability of solution culture and greenhouse screening methods. Crop Sci. 30:1049-1054.

Carter, T.E., Jr., and T.W. Rufty. 1993. Soybean plant introductions exhibiting drought and aluminum tolerance. p. 335-346. *In* C.G. Kuo (ed.) Adaptation of food crops to temperature and water stress: proceedings of an international symposium, Taiwan. 13-18 Aug. 1992. Publi. no. 93-410. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Chapin, F.S. 1980. The mineral nutrition of wild plants. Annu. Rev. Ecol. Syst. 11:233-260.

CIAT. 2008. Improved beans for the developing world. Outcome line SBA-1. Annual report 2008. Cali, Colombia.

CIAT. 2005. Bean Improvement for the tropics. Project IP-1, Annual report 2005. Cali, Colombia.

Clarkson, D.T. 1965. The effect of aluminum and some trivalent metal cations on cell division in the root apices of *Allium cepa*. Ann. Bot. 29:309-315.

Delhaize, E., and P.R. Ryan. 1995. Aluminum toxicity and tolerance in Plants. Plant Physiol. 107:315-321

Delhaize, E., S. Craig, C.D. Beaton, R.J. Bennet, V.C. Jagadish, and P.J. Randall. 1993. Aluminum tolerance in wheat (*Triticum aestivum* L.). I. Uptake and distribution of aluminum in root apices. Plant Physiol. 103:685-693.

Devine, T.E., C.D. Foy, D.L. Mason, and A.L. Fleming. 1979. Aluminum tolerance in soybean germplasm. Soybean Genet. Newsl. (Ames) 6:763-782.

Eisenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15:763-782.

Foy, C.D. 1988. Plant adaptation to acid, aluminum-toxic soils. Commun. Soil Sci. Plant Anal. 19:959-987.

Foy, C.D., A.L. Fleming, and W.J., Armiger. 1969. Aluminum tolerance of soybean varieties in relation to calcium nutrition. Agron. J. 61:505-511.

Foy, C.D., J.A. Duke, and T.E. Devine. 1992. Tolerance of soybean germplasm to an acid tatum subsoil, J. Plant Nutr. 15:527-547.

Girdthai, T., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook, and A. Patanothai. 2010. Relationship between root characteristics of peanut in hydroponics and pot studies. Crop Sci. 50:159-167.

Horst, W.J., and F. Klotz. 1990. Screening soybean for aluminum tolerance and adaptation to acid soils. p.355-360. *In* N. El Bassam et al. (ed.) Genetic aspects of plant mineral nutrition. Kluwer Acad. Publ., Dordrecht, the Netherlands.

Johansen, C., B. Baldev, J. B. Brouwer, W. Erskine, W. A. Jermyn, L. J. Lang, B. A. Malik, A. A. Miah, and S. N. Silim. 1994. Biotic and abiotic stresses constraining productivity of cool season food legumes in Asia, Africa and Oceania. p.175-194. *In* Muehlbauer, F. J. and W. J. Kaiser (ed.) Expanding the Production and use of cool season food legumes. Kluwer, Academic Publishers, Dordrecht, The Netherlands

Kopittke, P.M., N.W. Menzies, and F.P.C. Blamey. 2004. Rhizotoxicity of aluminate and polycationnic aluminum at high pH. Plant Soil. 177-1866.

Liu, L., Y. Gan, R. Bueckert, K.V. Rees, and T. Warkentin. 2010. Fine root distributions in oilseed and pulse crops. Crop Sci. 50:222-226.

Lambers, H., O.W. Nagel, and J.J.C.M. van Arendonk. 1995. The control of biomass partitioning in plants from “favourable” and “stressful” environments: a role gibberellins and cytokinins. Bulg. J. Plant Physiol. 21 (2-3):24-32.

Lambers, H., and H. Poorter. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological causes and ecological consequences. Adv. Ecol. Res. 23:87-261.

Little, R. 1988. Plant soil interaction at low pH: Problem solving genetic approach. Commun. Soil Sci. Plant Anal. 19:1239-1257.

López-Marín, H.D., I.M. Rao, and M.W. Blair. 2009. Quantitative trait loci for aluminum toxicity resistance in common bean (*Phaseolus vulgais* L.). Theor. Appl. Genet. 119: 449-458.

Lynch, J. 1995. Update on root biology: Root architecture and plant productivity. Plant Physiol. 109:7-13.

Manrique, G., I. Rao, and S. Beebe. 2006. Identification of aluminum resistant common bean genotypes using a hydroponic screening method. Paper presented at the 18th World Congress of Soil Science, Philadelphia, USA, July 9-15, 2006.

Marschner, H. 1991. Mechanisms of adaptation of plants to acid soils. Plant Soil 134:1-20

Massot, N., M. Llugany, Ch. Poschenrieder, and J. Barcelo. 1999. Callose production production as indicator of aluminum toxicity in bean cultivars. J. Plant Nutr. 22:1-10.

Mossor-Pietraszewska, T. 2001. Effect of aluminum on plant growth and metabolism. Acta Biolochimica Polonica 48(3):673-686.

Mugai E.N., S.G. Agong, and H. Matsumoto. 2000. Aluminum tolerance machanisms in *Phaseolus vulgaris* L.: citrate synthase activity and TTC reduction are well correlated with citrate secretion. Soil Sci. Plant Nutr. 46:939-950.

Muñoz-Perea, C.G., H. Terán, R.G. Allen, J.L. Wright, D.T. Westermann, and S.P. Singh. 2006. Selection for drought resistance in dry bean landraces and cultivars. Crop Sci. 46:2111-2120.

Narasimhamoorthy, B., E.B. Blancaflor, J.H. Bouton, M.E. Payton, and M.K. Sledge. 2007. A comparaison of hydroponics, soil, and root staining methods for evaluation of aluminum tolerance in *Medicago truncatula* (Barrel medic) germplasm. Crop Sci. 47:321-328.

Pandey, S., H. Ceballos, R. Mgnavaca, A.F.C. Bahia Filho, J. Duque-Vargas, and L.E. Vinasco. 1994. Genetics of tolerance to soil acidity in tropical maize. Crop Sci. 34:1511-1514.

Polanía, J., I.M. Rao, S. Beebe, and R. García. 2009. Desarrollo y distribución de raices bajo estrés por sequía en frijol común (*Phaseolus vulgaris* L.) en un sistema de tubos con suelo. Agron. Colombiana 27: 25-32.

Rangel, A.F., M. Mobin, I.M. Rao, and W.J. Horst. 2005. Proton toxicity interferes with the screening of common bean (*Phaseolus vulgaris* L.) genotypes for aluminum resistance in nutrient solution. J. Plant Nutr. Soil Sci. 168:607-616.

Rangel, A.F., I.M. Rao, and W.J. Horst. 2007. Spatial aluminum sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes with contrasting aluminum resistance. J. Exp. Bot. 58:3896-3904.

Rangel, A.F., I. M. Rao, H.P. Braum, and W. J. Horst. 2010. Aluminum resistance in common bean (*Phaseolus vulgaris* L.) involves induction and maintenance of citrate exudation from root apices. Physiol. Plant. 138:176-190.

Rao, I. M. 2001. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. p.583-613. *In* Pessarakli, M. (ed.) Handbook of Plant and Crop Physiology. Marcel Dekker, Inc., New York, USA.

Rhue, R.D., and C.O. Grogan. 1977. Screening corn for Al tolerance using different Ca and Mg concentration. Agron. J. 69:755-760.

Rizhsky, L., H. Liang, and R. Mittler. 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol. 130:1143-1151.

Ryan, P.R., J.M. DiTomaso, and L.V. Kochian. 1993. Aluminum toxicity in roots: An investigation of spatial sensitivity and the role of the cap. J. Exp. Bot. 44:437-446.

Ryser, P., and H. Lambers. 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil 170:251-265.

Samac, D.A., and M. Tesfaye. 2003. Plant improvement for tolerance to aluminum in acid soils – a review. Plant Cell, Tissue and Organ Culture 75:189-207.

Sapra, V.T., T. Mebrahtu, and L.M. Mugwira. 1982. Soybean germplasm and cultivar aluminum tolerance in nutrient solution and Bladen clay loam soil. Agron. J. 74:687-690.

Shen, H., X. Yan, X. Wang, and S. Zheng. 2002. Exudation of citrate in common bean in response to aluminum stress. J. Plant Nutr. 25:1921-1932.

Silva, I.R., T.J. Smyth, D.W. Israel, and T.W. Rufty. 2001. Altered aluminum inhibition of soybean root elongation in the presence of magnesium. Plant Soil 230:223-230.

Singh, S.P., and J.W. White. 1988. Breeding common beans for adaptation to drought conditions. p.261-285. *In* White, J. W., F. Hoogenboom, F. Ibarra, and S.P. Singh (Eds.) Research on Drought Tolerance in Common Bean. Working Document No. 41, Bean Program, CIAT, Cali, Colombia.

Spehar, C.R. 1994. Aluminum tolerance of soya bean genotypes in short term experiments. Euphytica 76:73-80.

Sponchiado, B.N., J.W. White, J.A. Castillo, and P.G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp. Agric. 25:249-257.

Subbarao, G.V., C. Johansen, A.E. Slinkard, R.C. Nageswara Rao, N.P. Saxena, and Y.S. Chauhan. 1995. Strategies for improving drought tolerance in grain legumes. Crit. Rev. Plant Sci. 14:469-523.

Thung, M., and I. M. Rao. 1999. Integrated management of abiotic stresses. p.331-370. *In* Singh, S. P. (Ed) Common Bean Improvement in the Twenty-first Century. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Urrea-Gómez, R., E. Ceballos, S. Pandey, A.F.C. Bahía filho, and L.A. León. 1996. A greenhouse screening technique for acid soil tolerance in maize. Agron. J. 88:806-812.

Villagarcia, M.R., T.E. Carter ,Jr., T.W. Rufty, A.S. Niewoehner, M.W. Jennette, and C. Arrellano. 2001. Genotypic ranking for aluminum tolerance of soybean roots grown in hydroponics and sand culture. Crop Sci. 41:1499-1507.

White, J. W., and J. A. Castillo. 1988. Studies at CIAT on mechanisms of drought tolerance in common bean. p. 146-151. *In* White, J. W., G. Hoogenboom, F. Ibarra, and S. P. Singh. (Eds.) Research on drought tolerance in common bean, Centro Internacional de Agricultura Tropical, Cali, Colombia.

Wolters, H., and G. Jürgens. 2009. Survival of the flexible: hormonal growth control and adaptation in plant development. Nat. Rev. Genet. 10:305-317.

Wortmann, C.S., R.A., Kirkby, C.A. Eledu, and D.J. Allan. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT publication no. 297, CIAT, Cali, Colombia.

Yang, Z., D. Eticha, I. M. Rao and W. J. Horst. 2010. Alteration of cell-wall porosity is involved in osmotic stress-induced enhancement of aluminium resistance in common bean (*Phaseolus vulgaris* L.). J. Exp. Bot. (in press).

Figure captions

Figure 1. Tap root elongation rate (TRER) under high Al (20 µM Al) (A), and per cent Al-induced inhibition of tap root elongation rate (B) and increase of root mean diameter (C) of 11 bean genotypes under hydroponic screening with two levels of Al (20 µM Al and 0 µM Al) at pH 4.5. Bars represent means ± SD, with 4 replicates. Different letters indicate differences between at $P < 0.05$ (REDGRQ test).

Figure 2. Relationship between Al induced inhibition of tap root elongation rate and increase of mean root diameter of 11 bean genotypes grown for 48 h in a nutrient solution containing 5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 at pH 4.5 under hydroponic screening with two levels of Al (20 µM Al and 0 µM Al).

Figure 3. Relationship between total root length of 11 bean genotypes in Al treatment in hydroponics and soil tube evaluation, and controls for the two screening methods. The Al treatment was characterized by a soil with 76% Al soil saturation (pH = 4.1) for top-soil of the cylinder (0-10 cm), and 83% saturation for subsoil (10-75 cm) (pH = 4.14). The low Al saturation was made by tubes packed for topsoil with soil (28 % Al saturation, pH = 4.45) and for subsoil (58 % Al saturation, pH = 4.29). Hydroponic evaluation was done in a nutrient solution containing 20 µM Al (with 0 µM Al for the control). The roots of seedlings were harvested after 5 days of Al treatment.

Figure 4. Relationship between total root length and shoot attributes of 11 bean genotypes under individual and combined stress of Al and drought. The Al treatment was characterized by a soil with 76% Al soil saturation and pH of 4.1 for top-soil of the cylinder (0-10 cm), and 83% saturation for subsoil (10-75 cm) with pH 4.14. The low Al saturation was made by tubes packed for topsoil with soil (28 % Al saturation, pH 4.45) and for subsoil (58 % Al saturation, pH 4.29). 29 days-old plants were harvested for the Al stress experiment. Water stress was imposed to plant after 10 days of initial growth for the third experiment (Individual and combined stress of Al and drought) and 23 days-old plants were harvested.

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3. Phenotypic evaluation of interspecific Recombinant Inbred Lines (RILs) of *Phaseolus* species for their resistance to aluminum and tolerance to aluminum-toxic acid soil under greenhouse conditions

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Abstract: Common bean is the most important cultivated grain legume in the world for direct human consumption. Aluminum (Al) toxicity is among major abiotic constraints limiting bean production in acid soil regions of the tropics. To improve the resistance of common bean to Al, progenies of Al sensitive *Phaseolus vulgaris* (SER16) and an Al resistant *Phaseolus coccineus* (G35346-3Q) including 94 $F_{5:6}$ Recombinant Inbred Lines (RILs) of the cross SER16 x (SER16 x G35346-3Q) were characterized for their resistance to Al and tolerance to Al-toxic acid soil under greenhouse conditions using hydroponic and soil tube systems. In each experiment a randomized complete block design with 3 replications was used. Two levels of Al (0 and 20 µM), were employed for phenotypic evaluation in the hydroponic system while an oxisol from Santander of Quilichao (Colombia) with low Al (12.5%; pH 4.6) and high Al-saturation (77 %; pH 4.1) was used for evaluation with soil tube (75 cm soil depth) system. For the low Al saturation as a control treatment, soil was adequately fertilized. Seedlings of all 102 genotypes (94 RILs, 2 parents and 6 checks) except the donor parent (G35346-3Q) showed reduced root elongation rates under the 20 µM Al treatment in the hydroponic system. Sensitive genotype VAX1 exhibited greatly reduced root growth rate by 69.5% whereby the most Al-resistant lines were G35346-3Q, and RILs ALB32, ALB41, ALB45 and ALB23 with primary root elongation rates reduced by -15.7%, 2.6%, 3.5%, 5.4%, and 8.1%, respectively. The root growth rate of an Andean genotype ICA Quimbaya was reduced by 12.5%. In the soil tube system, more than 50% of the RILs were deeper rooted than the Al sensitive parent SER16. Correlation between leaf area and total root length was highly significant under both high ($r = 0.70$, $P < 0.001$) and low ($r = 0.56$, $P < 0.001$) Al saturation. Genotypic ranking for Al resistance was different between the hydroponic and soil tube systems indicating that the genotypes that are Al resistant are not necessarily tolerant to acid soil conditions. Phenotypic evaluation using both systems allows identifying genotypes with Al resistance combined with acid soil adaptation for both shoot and root vigor. The results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

Abbreviations: TRER 24h: Tap root elongation rate between 0 to 24 hours of exposure to with and without Al in solution; TRER 24-48h: Tap root elongation rate between 24 and 48 hours of exposure to with and without Al in solution; TRER 48h: Tap root elongation rate between 0 to 48 hours of exposure to with and without Al in solution; TRL: Total root length; MRD: Mean root diameter; RTP: Number of root tips; SRL: Specific root length; RDP at 34d: Root depth at 34 days; Larea: Leaf area; SDBW: Shoot dry biomass weight; R:Sh ratio: Root:Shoot ratio

Introduction: Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct human consumption worldwide. Annual production, including both dry and snap bean, exceeds 21 million metric tons (Miklas et al., 2006). A majority of the bean production occurs under low input agriculture on small-scale farms in developing countries particularly in Latin America and Africa. The most productive soils in this part of the world are already under cultivation, and those available for agricultural expansion are often strongly acid and possess toxic levels of soil aluminum (Al) saturation (Kamparath, 1984).

Aluminum toxicity in acid soils is one of the major constraints to crop production worldwide (Rao et al., 1993; Shen et al., 2004). It is a potential growth-limiting factor for plants (Foy, 1992; Foy, 1996) and constitutes the third most abundant element in the earth's crust and a formidable phytotoxic barrier to crop production in acidic soils which represent 40% of the world's arable lands (Kochian, 1995). Al is present in all soils, but its toxicity is manifested only in acidic conditions, in which the phytotoxic form Al^{3+} predominates (Rout et al., 2001). Soils with high Al-saturation are often associated with a complex of factors (including Ca and P deficiency and Mn toxicity) that affect the ranking of genotypes for Al tolerance (Campbell and Carter, 1990).

Common bean proved to be very sensitive of low pH (4.3), with large genotypic differences in proton sensitivity (Rangel et al., 2005). The low pH in acid soils itself is not so much the cause of problems, but the fact that the solubility of specific metals (such as Al) depends on pH (Kochian et al., 2005). When the soil pH is lower than 5.0, Al is solubilized in the soil solution and absorbed by plant roots. Inhibition of root elongation has been widely recognized as the most striking symptom of Al toxicity on plant (Clarkson 1965, Foy, 1988). Al sensitivity is located specifically at the root apex and absorbed Al inhibits root elongation severely within an hour(s). Al-sensitive plants absorb more Al than do Al-resistant plants (Rangel et al., 2009), and thus the exclusion mechanism of Al is thought to be the major mechanism for

Al resistance (Matsumoto, 2000). It has been shown that the toxic effects of Al in soil can be overcome by adding appropriate amendments to acid soil (Pandey et al., 1994). Lime application is considered as a short term solution but it is not affordable to most households in developing countries that grow beans.

Mechanisms of Al toxicity and resistance are complicated and have not yet been fully characterized (Ryan and Delhaize, 2010; Horst et al., 2010). Genotypic and phenotypic differences for Al resistance exist among plant species including beans (Rangel et al., 2005) but reliable ranking in field for acid soil tolerance is still a problem for breeders.

Plant resistance to Al stress is a key component of an appropriate and effective integrated approach for farmers with low income in Africa and Latin America. Along with diverse germplasm and an appropriate breeding program, a reliable screening procedure for Al stress is one of the most important tools required to effectively develop Al-resistant cultivars **(**Narasimhamoorthy et al., 2007). In maize, Quantitative trait locus (QTL) analysis for Al tolerance with a set of recombinant inbred lines (RILs) showed that some of the RILs were considerably more Al tolerant than their parents (Hoekenga et al., 2003; Kochian et al., 2004). Total root length, surface area, and branching patterns have been shown to influence nutrient uptake (Raper et al., 1978). Despite the existence of many Al tolerance screening methods, the use of a single method to identify Al tolerant genotypes may lead to misleading results due to the complexities involved in each method **(**Narasimhamoorthy et al., 2007).

Previous research at CIAT has shown that some accessions of *Phaseolus coccineus* are more resistant to Al in solution than common bean (CIAT, 2005; Butare et al., 2010). Significant genotypic differences in Al resistance in common bean were also reported based on Al-inhibited root elongation in nutrient solution (Massot et al., 1999; Rangel et al., 2005; Manrique et al., 2006; Rangel et al., 2007; López-Marín et al., 2009). Breeding for resistance to Al toxicity is therefore an alternative that should be explored.

Nutrient solution cultures to simulate acid soil solutions were used by many plant physiologists in screening for Al resistance (Wenzl et al., 2003). These methods of assessing root growth in nutrient solutions are more attractive than soil assays (Villagarcia et al., 2001), as they provide controlled forms of Al toxicity and are easily repeatable. The hydroponic system is relatively rapid to perform and many plants can be evaluated in a short time and in a small space. Few breeders have adopted hydroponic screening system because it is usually limited to seedling assays (Villagarcia et al., 2001); but it has been used widely to screen cultivars of soybean for Al resistance (Sartain and Kamprath, 1978; Horst et al. 1992, 1997).

The best option for plant breeders whenever possible will be to conduct screening in the target field. However, in practice, reliable ranking of genotypes in the field can be difficult, because exchangeable Al levels may not be uniform and because environmental factors interact with soil Al to mask the expression of Al-toxic acid soil tolerance (Goldman et al., 1989; Campbell and Carter, 1990). Greenhouse soil-based rankings for Al resistance could be soil-type dependent and thus may not be easily reproducible across wide geographical areas. However, use of a combination of hydroponic and greenhouse soil-based evaluations could identify acid soil tolerant genotypes. At CIAT, a technique using plastic tubes with high Al saturation soil was developed to rank genotypes for Al-toxic acid soil tolerance based on differences in root development and distribution (Butare *et al*., 2010). The technique allows evaluation of effects of Al toxicity on plant growth (shoot and root development) in conditions similar to field. The methodology is reproducible since it employs a known acid soil with high Al saturation from the target area. It permits characterizing the depth of the primary root penetration, extraction of roots for quantification of root distribution across soil depth and total root length, and the degree of root branching in soil with a uniform bulk density. Computer-assisted electronic image analyses have made root analysis less time-consuming and allowed more accurate and less subjective measurement of root characteristics than the human eye is capable of making (Collins et al., 1987; Cunningham et al., 1989; Stutte and Stryjewski, 1995; Box, 1996, 2001).

The objective of this research work was to conduct phenotypic evaluation of a population of recombinant inbred lines (RIL) of *Phaseolus* species using hydroponic and soil tube systems and make a comparative analyses of these two methods to identify Al resistant and acid soil tolerant genotypes based on root and shoot traits.

Materials and Methods

Plant Material: An accession of *P. coccineus*, G35346-3Q, a sister species of common bean which is characterized by a very aggressive vine with great biomass and low harvest index; and a Mesoamerican small red drought tolerant common bean line SER16 were identified as contrasting parents (Butare *et al*., 2010). A high level of Al resistance had been observed in G35346-3Q compared to SER16 (CIAT, 2007;

Butare *et al*., 2010). A backcross of the F_1 hybrid to the recurrent common bean parent was pursued to recover the desirable plant and seed type of *P. vulgaris*. Ninety-four RILs from the $F_{5:6}$ generation of SER16 x (SER16 x G35346-3Q) were developed by single seed descent. This study was conducted using 102 bean genotypes including the 94 RIL, both parents, and four checks from the Mesoamerican gene pool (VAX1, Tio-Canela75 and DOR390 and G21212); and an elite Andean cultivar (ICA Quimbaya); and one *Phaseolus acutifoius* accession (G40159). Twenty five seeds (fifteen for hydroponics and ten for soil tubes) from each bean material were surface sterilized by sodium hypochlorite (1%) for five minutes; rinsed with abundant distilled water and pre-germinated in a sandwich system using filter paper and Styrofoam soaked with tap water in an upright position (Rangel et al., 2007) for 48 hours. For evaluation in nutrient solution, germinated seedlings were maintained in sandwiches for 24 hours more to allow further root development before transferring them to tanks containing the nutrient solution (Butare *et al*., 2010). For the soil tube system, uniform seedlings with emerged radicals were transplanted to soil tubes (one seedling per tube) in the center of each cylinder (Butare *et al*., 2010).

For purposes of this paper, Al resistance refers to the reaction of genotype to toxic Al in the hydroponic system, whereby tolerance to Al-toxic acid soil refers to tolerance to high Al saturation in acid soil conditions characterized with low nutrient availability (Butare *et al*., 2010).

Phenotypic evaluation in hydroponic system: Ninety-eight bean genotypes including 90 RIL, the two parents and six controls were evaluated under hydroponic system. After germination, seedlings with well developed uniform roots were transferred to a tray floating in nutrient solution (López-Marín et al., 2009; Butare et al., 2010) before applying Al treatment (0 or 20 µM $AlCl_3$). The number of replications was variable among genotypes (from 3 to 7). Monitoring of the pH was done each 6 hours and maintained at 4.5. The greenhouse day/night temperature was on average 29.5/22.8°C; relative air humidity of 50.4/72.6% (day/night); and the maximum photosynthetic photon flux density at noon was 1100 µmol m^{-2} s^{-1}. Al resistance was assessed by measuring root elongation of tap roots after 24 (TRE at 24h) and 48 hours (TRE at 48h) with a ruler from a point initially marked at 3 cm behind the root apex, to calculate root growth rate and inhibition of root elongation (Rangel et al., 2007). At harvest, roots were separated from shoots and transferred to plastic bags and refrigerated at 4°C while root image analysis was carried out using a flatbed color scanner "Epson Expression 1680 Scanner". Root attributes, including total root length (TRL), mean root diameter (MRD), and number of root tips (RTP) were quantified using a computerized software program WinRhizo®. The change in root mean diameter, specific root length (SRL), proportion of fine roots and root growth inhibition (RGI) were calculated. Root dry biomass weight (RDBW) for each plant was determined after drying the entire root system in an oven at 60°C for 48 hours.

Phenotypic evaluation in soil tube system: A greenhouse evaluation to quantify phenotypic differences among 94 RILs was carried in transparent plastic cylinders at two levels of Al saturation (Butare et al., 2010). Soil was collected from Santander of Quilichao, Department of Cauca in Colombia (Lat. 3° 06' N; Long. 76° 31' W; Altitude 990 m) at soil layer of 0-20 cm from surface. The soil was characterized as an Oxisol (very fine kaolinitic, isohypothermic, plinthic Kandiudox) with pH of 4.1, bulk density of 1.13 $g.cm^{-3}$ and high Al saturation of 77%. Soil for the low Al treatment presented pH of 4.6, bulk density of 1.26 $g.cm^{-3}$ and Al saturation of 12%. The low Al soil as control treatment was fertilized with adequate level of nutrients (kg ha^{-1}): 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo while the high Al soil was not supplied with any additional nutrients to simulate low fertility acid soil stress.

The experiment was planted as a Randomized Complete Block Design (RCBD) with 3 replications. Plants were grown in soil tubes (80 cm long and 7.5 cm diameter with 75 cm soil depth) inserted into PVC pipes. Each cylinder was filled uniformly with soil (5.01 kg for low Al saturation treatment and 4.76 kg for high saturation). Soil moisture was maintained at 80% of field capacity by weighing each soil tube and applying water to the top of each tube to compensate the difference in weight. The experiment was conducted in a greenhouse at an average temperature of 29.5/22.8°C (day/night), relative air humidity of 43.7/60.9% (day/night), and a maximum photosynthetic photon flux density of 1100 µmol m^{-2} s^{-1}. Plants were harvested at 34 days after planting as described previously (Butare et al, 2010).

Shoot and Root attributes: Shoot parameters including chlorophyll content, leaf temperature and number of leaves were measured on plants in the soil tube experiment. Total chlorophyll content (SPAD) was measured every week using a SPAD-502 chlorophyll meter (Minolta camera Co., Ltd, Japan). At harvest, leaf area of 34 day-old plants was determined by scanning leaves of each genotype using a LI-3100 leaf area meter (LI-COR Biosciences, USA). Shoot dry biomass weight (SDBW) was measured after drying leaves, stems and pods in an oven at 70°C for 72 hours. Root attributes were determined by image

analysis with the WinRhizo software program (Regent Instruments INC., Canada): total root length, mean root diameter, root volume, root length by category of root diameter (<0.5, 0.5-1.0 mm). It was not possible to determine tap root growth rate and number of root tips in the soil tube experiment.

Statistical Analysis: PC SAS was used for all statistical analysis. Analysis of variance was performed using the ANOVA procedure (PROC GLM) of statistical program SAS 9.1 (SAS institute Inc. SunOS 5.9 platform). Correlation coefficients were calculated by the PROC CORR procedure using means across all treatments and replications for the two experiments. Significant differences (*, **, and ***) were detected at the 0.05, 0.01, and 0.001 probability levels, respectively. Relationships between the two Al-screening methods, genotypes characteristics (root and shoot traits) were determined. Differences between genotypes were analyzed with the least significant difference (LSD) at $P < 0.05$, and means of each dependent variable (root and shoot characteristics) were compared using the Ryan-Einot-Gabriel-Welsh Multiple Range Test (REGWQ test).

Results

Mean for some specific root and shoot traits in both Al treatment and control for the two parents G35346-3Q and SER16 have been compared, and the implication for inheritance of these traits from each parent was identified (Table1). The range of progeny means exceeded that of their parents both in hydroponics (with and without Al) and soil tube screening (with high and low Al saturation in soil) suggesting transgressive segregation for these plant phenotypes.

Table1. Parents of interspecific populations G35346-3Q and SER16 evaluated under Al stress and control test in hydroponics and soil tube screening, and range of recombinant inbred lines for specific plant traits.

	Al treatment				Control test			
	G35346-3Q	**SER16**	**Range**	**$LSD_{0.05}$**	**G35346-3Q**	**SER16**	**Range**	**$LSD_{0.05}$**
Hydroponics								
TRE24h	1.43	1.38	0.96-1.98	0.15	1.33	1.68	1.33-2.18	0.19
TRE24-48h	1.53	0.95	0.03-1.67	0.25	1.2	1.67	1.06-2.07	0.27
TRE48h	1.47	1.15	0.57-1.8	0.16	1.27	1.67	1.24-2.03	0.2
TRL	19.02	21.86	4.37-29.74	28.38	28.18	38.87	10.12-50.7	41.15
MRD	0.62	0.41	0.39-0.69	0.03	0.58	0.35	0.32-0.58	0.03
RTL	171.67	440.83	52-508.25	4.76	340	1066.7	187.8-1545.5	7.98
SRL	834.5	1276.3	834.5-1691	454.39	1101	1497.7	1101-2818.7	297.46
Soil tube								
TRL	42.9	16.61	8.81-42.9	13.17	79.4	64.8	36.13-97	41.8
MRD	0.46	0.40	0.30-0.47	0.12	0.43	0.32	0.29-0.68	0.18
SRL	62.09	72.13	51.29-95.8	27.87	60.25	98.15	60.25-143.07	39.7
RDP34d	64.33	37.4	25.17-64.3	21.58	57.3	67.5	43.7-75	25.7
Larea	259.2	105.94	48.8-271	146.3	443.6	548.2	268.8-853.9	322
SDBW	1.25	0.81	0.26-1.43	0.63	2.6	3.46	1.38-5.2	1.95
R:Sh ratio	0.59	0.29	0.14-1.06	0.44	0.54	0.21	0.11-0.54	0.17

Phenotypic differences in Al resistance in hydroponic system

Tap Root Growth Rate and Al resistance: Al resistance screening using hydroponic system with two levels of Al (0 and 20 µM Al) was used to detect genotypic differences in Al resistance. Analysis of variance revealed highly significant genotype (RIL) effects, and genotype x Al ($P < 0.001$) interaction, indicating differential response of the 90 RILs in tap root growth rate to Al stress (Table 2) at 0-24 h, 24-48h and 0-48h. Mean value for tap root growth rate in nutrient solution screening was 1.18 mm h^{-1} for Al treatment (20 µM Al) and 1.71 mm h^{-1} for the control (0 µM Al) treatment. Seedlings of all 102 genotypes (except the donor parent G35346-3Q) under Al stress in nutrient solution showed that root elongation

rates were affected by the supply of 20 µM Al-stress. Sensitive genotypes, ALB79, ALB77, ALB18, ALB49, ALB95, ALB10, ALB13, ALB94, ALB71 and VAX1, exhibited root-elongation rate per hour greatly reduced by 48.3- 69.5%. Ten genotypes were Al-resistant with less tap root elongation rate (TRER) reduction per hour. These were G35346-3Q, ALB32, ALB41, ALB45, ALB23, ALB43, ALB87, ALB78, ICA Quimbaya and ALB34 with root growth rate inhibition of -15.7%, 2.6%, 3.5%, 5.4%, 8.1%, 10%, 10.8%, 11.5%, 12.5% and 14.1%, respectively. SER 16 presented an inhibition of TRER of 31.1 %.

Table 2. Correlation between root characteristics of bean genotypes grown with 20 µM Al and mean squares (from combined ANOVA) of tap root elongation rate at 0-24 hours, at 0-48 hours, and between 24 and 48 hours; total root length (TRL), mean root diameter (MRD), number of root tips (RTP), and specific root length (SRL) for 98 bean genotypes including 90 RILs, 2 parents, 6 checks grown using hydroponic system.

Variable/Source	df	TRER 24h (mm)	TRER 48h	TRER 24-48h	TRL (m)	MRD (mm)	RTP (nb)	SRL ($m.g^{-1}$)
TRER 24h (mm)	_	1						
TRER 48h	_	0.86***	1					
TRER 24-48h	_	0.67***	0.94***	1				
TRL (m)	_	-0.16 (ns)	0.029 (ns)	0.05 (ns)	1			
MRD (mm)	_	0.38***	0.39***	0.34***	-0.37***	1		
RTP (nb)	_	-0.28**	-0.17 (ns)	-0.07 (ns)	0.83***	-0.65***	1	
SRL (m.g-1)	_	-0.09 (ns)	-0.03 (ns)	0.03 (ns)	0.40***	-0.69***	0.58***	1
Level of Al	1	1.6515***	3.9282***	7.8654***	137.4295***	0.1996***	10060.8242***	6410.1105***
Rep. (Al level)	12	0.0817***	0.1246***	0.2161***	4.4019***	0.0016***	74.3750***	270.6782***
Genotype	97	0.0325***	0.0426***	0.0733***	3.6138***	0.0040***	139.5238***	86.1822***
Genotype X Al level	97	0.0112***	0.0207***	0.0502***	0.2376 (ns)	0.0005***	16.9478***	58.3288***
Error	779	0.0064	0.0073	0.0146	0.268	0.0002	9.277	31.6438

*** Significant at the 0.001 probability level,
** Significant at the 0.01 probability level,
ns: not significant.

Relationships between TRER at 0-24h and TRER at 24-48h (Figure 1a), and TRER at 0-48h and total root length (TRL) (Figure 1b) in hydroponic system were analysed. Eight genotypes (ALB43, ICA Quimbaya, ALB45, ALB84, ALB87, ALB2, ALB23 and ALB106) were Al resistant by maintaining high TRER across these two separate time points (Figure 1a). ALB13, ALB18, ALB79 and ALB77 were found to be Al sensitive. Tap root growth rate at 0-24h significantly correlated to tap root growth at 24-48h ($r = 0.67$; $P<0.001$). TRER at 0-24h, 0-48h and 24-48h were highly correlated to mean root diameter (MRD) with significant but weak correlations, r = 0.38***, r = 0.39*** and r = 0.34***, respectively. Significant but weak negative correlation was found also between tap root growth rate at 24h and number of root tips (r = -0.28**).

The relationship between TRL and TRER at 24h was very weak ($R^2 = 0.0084$). ICA Quimbaya was the only genotype which combined high TRL (extensive root system) and high TRER at 24h while ALB18 and ALB104 were very sensitive to Al stress both with low TRL and TRER at 24h. ALB43 and ALB106 were observed to have high TRER accompanied by low TRL.

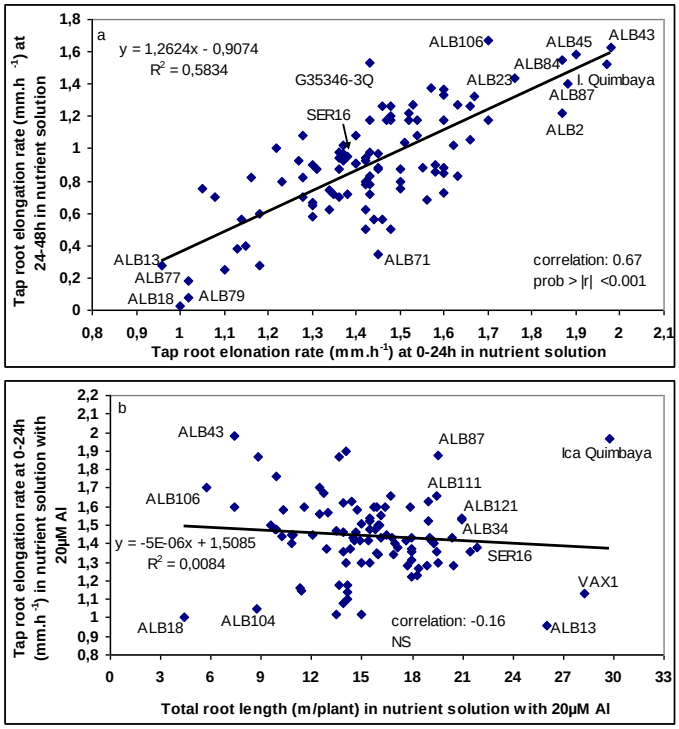

Figure 1. Relationship between tap root elongation rate at 0-24h and tap root elongation rate at 24-48h, and total root length (m) and tap root elongation rate at 0-24h of 98 bean genotypes including 90 RILs, two parents (G35346-3Q and SER16), and six checks (VAX1, DOR390, G21212, G40159, Tio canela75, and ICA Quimbaya) grown in nutrient solution containing 20 µM Al.

Superior root vigor and Al resistance in RILs: A highly significant genotype effect was found on total root length (TRL), but genotype x Al treatment interaction was not significant (Table 2). Effects on number of root tips (RTP) and mean root diameter (MRD) were highly significant ($P < 0.001$) for genotype (RIL), and genotype x Al treatment interaction (Table 2). The relationship between TRL and RTP, and between TRL and MRD is illustrated in Figure 2 for 98 bean genotypes under Al stress including 90 RIL, two parents and six check genotypes. TRL and RTP were highly correlated (Table 2) ($r = 0.83^{***}$). ICA Quimbaya, VAX1, ALB13, ALB88, SER16, ALB34 and ALB46 maximized the expression of these two traits and were characterized by an extensive root system with greater number of root tips. In contrast, ALB18, ALB99, ALB106 and ALB43 were more affected by Al-toxic stress in solution, without the ability to produce fine roots. A negative association was found between TRL and MRD ($r = -0.37^{***}$). VAX1, SER16, ALB46 and DOR390 presented high TRL and low MRD, with less increase in root diameter under Al stress. Average root diameter ranged from 0.32 to 0.58 mm without Al, and 0.39 to 0.69 mm under Al-stress (20 µM Al). ALB88 and ALB121 were also excellent with fine root systems (MRD < 0.45mm). Number of root tips (RTP) was negatively correlated to mean root diameter (MRD) ($r = -0.69^{***}$).

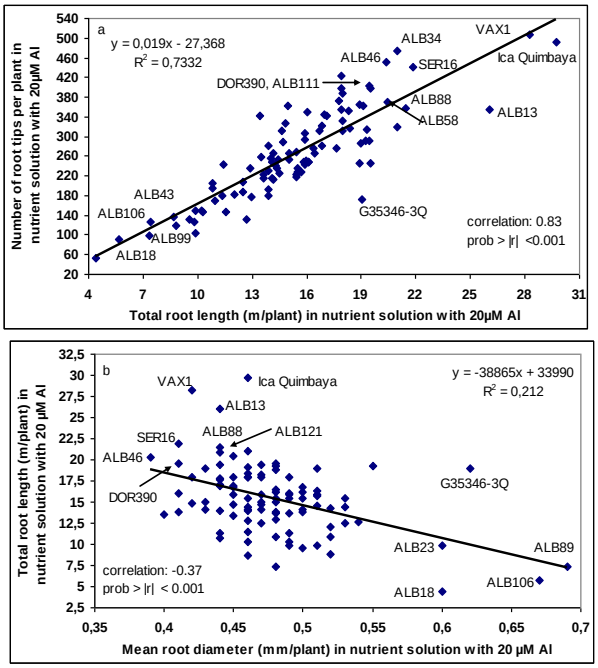

Figure 2. Relationship between total root length and number of root tips per plant, and total root length and mean root diameter per plant for 98 bean genotypes including 90 RILs, two parents (G35346-3Q and SER16), and six checks (VAX1, DOR390, G21212, G40159, Tiocanela75, and ICA Quimbaya) grown in nutrient solution containing 20 µM Al.

Phenotypic differences in acid soil tolerance in soil tube system

Rooting depth in soil: The major Al toxicity symptom observed in plants is inhibition of root growth that affects directly the distribution of roots in soil profiles. Rooting depth (RDP) of 34 day-old plants showed significant differences ($P < 0.001$) between the two levels of Al treatment, replications (Al level), genotype effects, and Genotype x Al interaction (Table 3). RDP of all bean genotypes were reduced in Al-stress except with G35346-3Q which showed deeper root penetration in high Al saturation soil tubes ($P<0.001$) than in low Al control. Mean value of RDP was 67.5 cm in the control treatment while it was 40.8 cm for high Al treatment in soil tubes. Reduction of RDP by Al stress for parents was -12% for Al resistant parent G35346-3Q and 43.5% for recurrent parent SER16. More than 50% of the RILs were deeper rooted than SER 16 which is considered as Al sensitive parent (REGWQ ranking). Significant difference was found between genotypes (at $P <0.05$, REGWQ test). REGWQ grouping revealed that rooting depth of resistant parent G35346-3Q, first in the group, showed significant difference ($P<0.05$) with the RDP of 29 genotypes that are considered as sensitive to Al-toxic acid soil. Quimbaya (second) was different only to 9 genotypes. Among recombinant inbred lines, ALB70 was significantly different ($P < 0.05$) with ALB106, ALB13 and DOR390; ALB65 and ALB72, statistically different to ALB13 and DOR390; and ALB was significantly different to DOR360. Al-toxic acid soil tolerance ranking based on the reduction of RDP classified the following genotypes as the most Al sensitive in acid soil: ALB13, DOR390, ALB60, ALB21, ALB95, ALB117, ALB106, ALB90, ALB56 and ALB110 with a decrease in root depth ranging between 52.2% and 64% when mean for low and high Al saturation was compared. Some genotypes showed no or little reduction of root growth on a percentage basis. These genotypes were headed by the resistant parent G35346-3Q (-12.2%), followed by VAX1 (-2.2%). Among the most deep rooted genotypes in high Al saturation were (in cm) G35346-3Q (64.3), ICA Quimbaya (60.2), ALB70 (57.7), ALB65 (56.8), ALB72 (56.8), ALB77 (54.33) and ALB91 (53.33). Rooting depth of ALB89 and ALB88 were respectively 49.5cm and 47cm, which were statistically equal to the best genotypes.

Table 3. Correlation between root and shoot characteristics of bean genotypes grown with high Al saturation and mean squares (from combined ANOVA) of total root length (TRL), mean root diameter (MRD), specific root length (SRL), and root depth (RDP) of 34 days-old plants, leaf area, shoot dry biomass weight (SDBW) and root:shoot ratio (R:Sh ratio) for 102 bean genotypes including 94 RILs, 2 parents, 6 checks grown using soil tube system.

Variable/Source	df	TRL (m)	MRD (mm)	SRL ($m.g^{-1}$)	RDP at 34d (cm)	Larea (cm^2)	SDBW (g)	R:Sh rate
TRL (m)	_	1						
MRD (mm)	_	-0.27***	1					
SRL ($m.g^{-1}$)	_	0.30**	-0.58***	1				
RDP at 34d (cm)	_	0.77***	-0.23*	0.14 (ns)	1			
Larea (cm^2)	_	0.70***	-'0.19 (ns)	0.15 (ns)	0.50***	1		
ShBiom (g)	_	0.60***	-0.07 (ns)	0.15 (ns)	0.40***	0.71***	1	
R:Sh rate	_	0.20*	-0.004 (ns)	-0.29**	0.30**	-0.08 (ns)	-0.48***	1
Level of Al	1	336.2426***	0,7980***	107.9268***	108.4536***	26966.814***	1108.818***	3.4845***
Rep. (Al level)	4	31.2301***	0,1405***	11.7738***	0.6383***	65.487***	2.775***	0,0116 (ns)
Genotype	101	0.3495***	0,0044**	0.3620***	0.1548***	18.964***	0,930***	0,0383***
Genotype X Al level	101	0.2233***	0,0033 (ns)	0.2331**	0.1312***	16.686***	0,673***	0,0207*
Error	404	0.1294	0.0031	0.1584	0.0758	8.423	0.283	0.0152

*** Significant at the 0.001 probability level
** Significant at the 0.01 probability level.
* Significant at 0.05 probability level
ns: not significant

Relationship between RDP and TRL in high Al soil saturation was determined. Genotypes characterized by an extensive and deep root system were G35346-3Q, ALB91, ALB77, ALB15, ALB40 and ALB88. ALB49 and ALB95 showed a poor root system, respectively 33.51 and 30.01 cm for root depth, and 8.81 and 9.5 m for total root length (Figure 3c). Root depth of the 34 day-old plants was highly correlated with some plant traits (Table 2) of soil tube experiment (High Al saturation): total root length (r = 0.77***), leaf area (r = 0.50***), and shoot dry biomass weight (r = 0.40***). There was also significant correlation with mean root diameter (r = -0.23*), and with root:shoot ratio (r = 0.30**).

The relationship of TRL with and without stress was quite different in hydroponics and in soil. A close relationship (R^2 = 0.84) was found between total root length with and without Al treatment in hydroponics (Figure 3a). Genotypes such as ICA Quimbaya, VAX1, ALB13, DOR390, and ALB88 maintained high total root length both with (20 µM Al) or without Al (0 µM Al). In contrast, the relationship between TRL in low Al and high Al saturation in soil tubes was weak (Figure 3b). Few genotypes showed good root development under both stress and non-stress conditions.

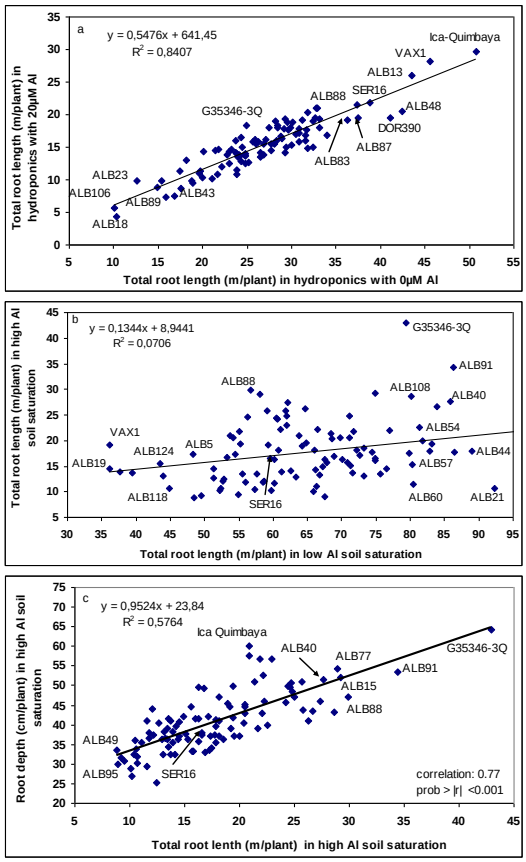

Figure 3. Relationships between total root length under hydroponics (0 and 20 µM Al), and between total root length in soil tube experiment with two level of Al saturation (low and high), total root length and root depth in soil with high Al saturation using soil tube system for 90 RILs, with 2 parents and 6 checks.

Interaction between root and shoot traits: Effects on Leaf area (Larea) and shoot dry biomass weight (SDBW) were highly significant for genotype, and genotype x Al treatment interaction (Table 3). Relationships between shoot attributes (Larea and SDBW) and root length density under high Al saturation in the soil tube experiment and the hydroponics screening were determined. Correlations between these two shoot traits and total root length under high Al saturation soil were highly significant, r = 0.70*** for leaf area and r = 0.60*** for shoot dry biomass weight (Table 3.). Al resistant genotypes, G35346-3Q, ALB91, ALB77, ALB89, ALB88, and ALB119 combined an extensive root system and high shoot development. In contrast, sensitive RIL ALB104, ALB106, ALB13 and ALB95 were poorly developed, producing less root and shoot biomass (Figure 5a). Leaf area was highly correlated to shoot dry biomass weight (r = 0.71***).

In the hydroponic system the number of root tips (RTP) and mean root diameter (MRD) were correlated to TRL, which was considered a key indicator of Al resistance at whole plant level. Therefore, relationships were investigated between RTP of plants grown in hydroponics and leaf area (Larea) of plants grown in soil tube (Figure 5b), and between MRD in hydroponics and shoot dry biomass weight (SDBW) in soil tubes (Figure 5c). The mean of MDR and RTP in hydroponics with Al-stress (20µM Al) were 0.48 mm and 270, respectively. In acid soil tube experiment, biomass allocation for leaf area ranged from 48.8 to 271 cm^2 and shoot biomass dry weight from 0.26 to 1.43 g. The relationship between RTP and Larea was poor (Figure 5b), and the correlation was not significant. Most genotypes with high number of root tips in hydroponics did not have extensive leaf area in soil tubes, although ALB88 and ALB58 combined good leaf area and higher number of root tips. The correlation between shoot biomass weight and mean root diameter was not significant. ALB77, ALB88, ALB117, ALB58, ALB15 and ALB38 were characterized by a fine root system and high shoot dry biomass weight under Al stress (Figure 5c.).

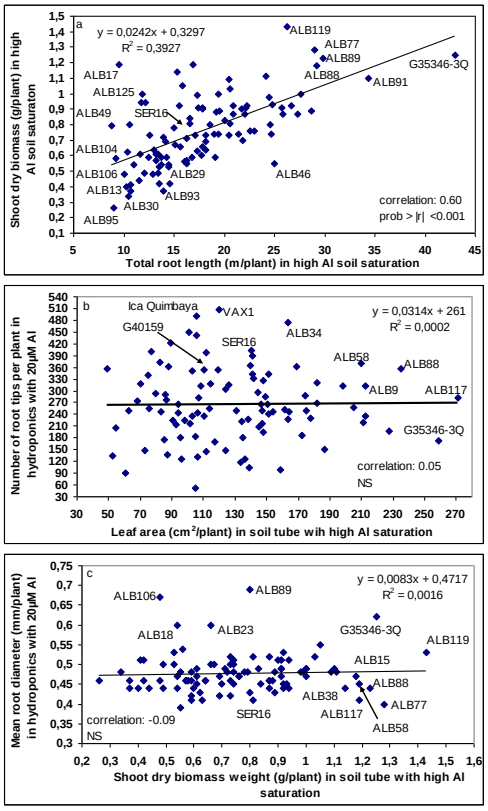

Figure 5. Relationship between root and shoot characteristics of bean genotype (Total root length, Number of root tips, Mean root diameter, Leaf area, and Shoot dry biomass weight per plant) of 98 genotypes including 90 recombinant inbred lines, two parents (G35346-3Q and SER16), and six checks (VAX1, DOR390, G21212, G40159, Tio canela75, and ICA Quimbaya) in hydroponics containing 20 µM Al and soil tube (with high Al saturation soil) screening method.

Correlations among treatments, traits and evaluation methods: The relationship between total root length in Al-toxic acid soil and total root length in hydroponics with Al (20 µM Al) was poor, and the correlation not significant. G35346-3Q, ALB88, ALB108, and ALB46 (Figure 4a) were among few genotypes that developed extensive root system in both evaluations.

As noted above, RTP and MRD in the hydroponic system were not correlated with leaf area and shoot dry biomass weight. A few weak correlations were found between root traits of seedlings evaluated in the two experiments (Table 4). Significant correlations were revealed between TRER at 24h in hydroponics and two root traits in soil tube (Table 3), specific root length (r = -0.25*) and R:S ratio (r = 0.21*). Total root length in hydroponics was correlated to MRD in soil tube (r = -0.29**; Figure 4b). Among the genotypes that showed good values of TRL in hydroponics and fine root system (lower values of MRD) in acid soil conditions were ALB88, ALB46, ALB67, ALB34 and ALB58. Mean root diameter in hydroponics also correlated with specific root length (SRL) in soil tube system (r = -0.24*). There was also significant correlation between root tips and two root traits in soil tubes, MRD (r = -38***; Figure 4c) and SRL (r = 0.26**). Genotypes that combined high number of root tips in hydroponics and fine root system in soil tubes included ALB46, ALB34, ALB54, ALB88, ALB67, and ALB58, several of which were identified as promising based on also other traits.

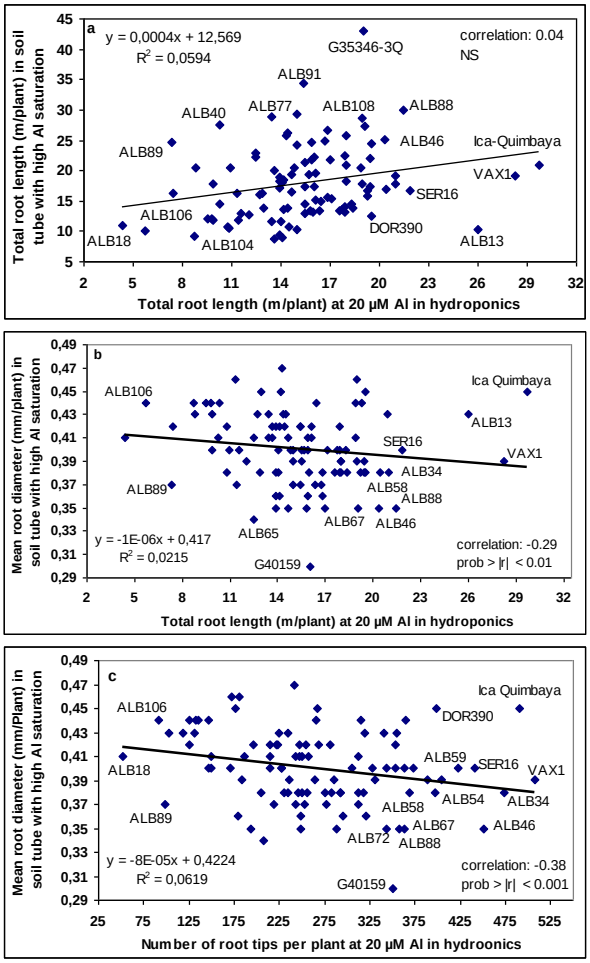

Figure 4. Relationship between root characteristics of bean genotype (Total root length, Mean root diameter, and number of rot tips in two greenhouse Al resistance screening methods, hydroponics (20 µM Al) and soil-based (high Al saturation) screening system.

Table 4. Correlation between root and shoot characteristics of bean genotypes including 90 RILs, 2 parents, and 6 checks grown under Al-stress using both hydroponics (20 µM Al) and soil tube screening (high Al saturation soil) systems.

Hydroponics / Soil tubes	TRL (m)	MRD (mm)	SRL (m.g^{-1})	RDP at 34d (cm)	Larea (cm2)	SDBW (g)	R:Sh rate
TRER 24h (mm)	0.04 (ns)	0.22 (ns)	-0.25*	-0.04 (ns)	-0.005 (ns)	0.001 (ns)	0.21*
TRER 48h	-0.002 (ns)	0.17 (ns)	-0.20 (ns)	-0.03 (ns)	-0.013 (ns)	-0.035 (ns)	0.20 (ns)
TRER 24-48h	-0.004 (ns)	0.14 (ns)	-0.15 (ns)	-0.02 (ns)	0.008 (ns)	-0.05 (ns)	0.13 (ns)
TRL (m)	0.04 (ns)	-0.29**	0.156 (ns)	0.017 (ns)	-0.024 (ns)	-0.04 (ns)	0.03 (ns)
MRD (mm)	-0.117 (ns)	0.16 (ns)	-0.24*	-0.02 (ns)	-0.058 (ns)	-0.09 (ns)	0.09 (ns)
RTP (nb)	0.099 (ns)	-0.38***	0.26**	0.06 (ns)	0.05 (ns)	0.005 (ns)	-0.025 (ns)
SRL (m.g^{-1})	0.094 (ns)	-0.15 (ns)	0.15 (ns)	-0.012 (ns)	0.103 (ns)	0.054 (ns)	-0.019 (ns)

*** Significant at the 0.001 probability level
** Significant at the 0.01 probability level.
* Significant at 0.05 probability level
ns: not significant

DISCUSSION

Root growth and aluminum resistance: A combination of hydroponics and soil-tube screening has been used to assess Al-resistance and acid soil tolerance among 102 bean genotypes. López-Marín et al. (2009) have showed with a mapping population on bean in hydroponic screening that genotype (RIL) effects, Al treatment, and the interaction RIL x treatment from the analyses of variance were highly significant for TRER, TRL, MRD, RTP, RDW and SRL. We found similar results also in our experiment except that Genotype x Al interaction for TRL was not significant (Table 2). At pH 4.3, Rangel et al., (2005) showed that root-elongation rates of SEA5 and VAX1 were reduced respectively by 74% and 85%. When comparing the Al-treatment to the control without Al in this study at pH 4.5, we found similar results whereby tap root growth of sensitive genotypes were affected by 79.5% (ALB79), 69% (ALB77), and 60.5% (ALB18). Al-induced changes in root elongation rates are paralleled by corresponding changes in the volume of the root cap (Bennet et al., 1987), suggesting that root growth may depend on maintaining minimum levels of activity (high rate of turnover of cap cells (Barlow, 1975) and links suggested between the production of cap cells and the quantity of polysaccharide material) in the cap (Bennet and Breen, 1989; Bennet and Breen, 1990).

In more detailed examination, the distal region of the transition zone (DTZ) was shown to be the most Al-sensitive root apical region for maize (Sivaguru and Horst, 1998). In common bean Al resistance was associated with activation and maintenance of an Al exclusion mechanism, especially in the transition zone but also in elongation zone (Rangel et al., 2007). Some of the differences observed in Al uptake in roots can be explained by differences in root growth since Al-tolerant roots continue to grow in the presence of Al and would effectively dilute the Al in apices (Delhaize et al., 1993a). Mean tap root growth rate in Al-toxic stress in the present study implied that for each hour there are more elongation of primary root cells in resistant RIL than in sensitive lines. Some RIL were outstanding in Al-resistance by maintaining a lower level of inhibition of root growth rates. As effect of Al on partial elongation of specific 1 mm root zones, Rangel et al. (2007) found that the maximum rate of relative root elongation was 3.9 mm h^{-1} (31%) in Quimbaya and 3.3 mm h^{-1} (41%) behind the root tip in VAX 1. We found that across all 98 genotypes the range of tap root elongation rate was between 0.57 and 1.8 mm h^{-1} at 3 cm behind the root tip in hydroponics screening with 20µM Al. In another experiment, Doncheva et al. (2005) concluded that Al-induced inhibition of root elongation rate, measurable after 45 min in the Al-sensitive maize variety HS 16X36, must be attributed to an Al-induced decrease of root cell expansion rather than to fast inhibition of root cell division.

Aluminum resistance ranking based on mean TRER between 24h and 24-48 classified ALB43, ICA Quimbaya, ALB45, ALB84, ALB106, ALB87, ALB23, ALB2, ALB32, ALB24, ALB56 and G35346-3Q as most resistant genotypes. A positive but mid-range correlation was found between TRER at 0-24h and TRER at 24-48h (r = 0.67***), suggesting that measurements of TRER at 0-24h may not be fully representative of TRER at 24-48h. The range of TRER decreased from 0.96-1.98 $mm.h^{-1}$ at 24h to 0.03-1.67 $mm.h^{-1}$ at 24-48h. Genotype ranking varied between the two periods of time indicating that different lines are responding differently. ICA Quimbaya was second for TRER at 0-24h and 6th for TRER at 24-48h, while G35346-3Q that was 53rd for TRER at 24h but 5th for TRER at 24-48h. G35346-3Q was the only genotype with greater TRER in the second period compared to the first, registering 1.43$mm.h^{-1}$ at the beginning (0-24h) and reaching 1.53$mm.h^{-1}$ between 24 and 48h. In their work on root development in maize, Bennet and Breen (1991) concluded that Al acts by releasing the root meristem from growth inhibition originating in the root cap. This could be an explanation of the high TRER of G35346-3Q at 24-48h that showed increase of root growth rate in the Al resistant parent. However, this trait was not readily transferred to the progenies through the single backcross that created the progenies.

Although inhibition of primary root growth could be associated to the crop's sensitivity to the stress, no association was found between TRER and TRL. Our results did not suggest that TRER has any effect on overall root system development (TRL), and therefore may not be useful for screening for acid soil tolerance. Although no association was found between TRER at 24h and TRL (20 µM Al), ICA Quimbaya showed Al resistance based on both of these root traits (Figure 1b). A weak and significant correlation was found between TRER at 24h, TRER at 24-48h, or TRER at 0-48h and average root diameter (r = 0.38***, 0.34*** and 0.39***, respectively), suggesting that differences in genotype ranking of the 90 RIL could be due to differences among genotypes in maintaining fine root (low MRD) development and high root growth rate, in addition to tolerance of Al stress.

Other investigators suggested that inhibition of cell elongation rather than cell division plays a role in short-term response to Al supply, whereas over longer periods of time (more than 24 h), both processes of cell elongation and cell division are inhibited (Matsumoto 2000, Stass et al., 2007). Bennet and Breen (1991) reported that the recovery of root growth rates from Al treatment are initially faster than normal suggesting that the early phases of recovery may involve growth stimulation.

Root Architecture and Aluminum Resistance: A clear difference in root length was observed between Al-tolerant and Al-sensitive seedlings of wheat at 20 µM Al (Delhaize et al., 1993). Villagarcia et al. (2001) showed that basal roots and branches from the taproot were clearly reduced in all genotypes of soybean. Al-toxicity is known to induce morphological changes on root architecture of sensitive genotypes. Root elongation of genotypes was inhibited as early as 1 h after the beginning of the Al treatment (Rangel et al., 2007). Fine roots are considered to be more important than thick roots in nutrient and water absorption, and therefore, more important in terms of Al tolerance (Eisenstat, 1992; Villagarcia et al., 2001; Liu et al., 2010). Inhibition of TRER was accompanied by an increase of MRD on Al-sensitive RIL (data not shown). Doncheva et al. (2005) showed that Al caused not only a reduction in the length of the main root, but also changes to the entire root architecture.

Two parents used in a mapping population of common bean, DOR364 and G19833 revealed a differences in MRD in response to Al-stress in nutrient solution (López-Marín et al., 2009), DOR364 with lower MRD value and G19833 with higher MRD value. Blancaflor et al. (1998) showed with maize that Al induced increase in root diameter over longer periods (> 4h). Our study revealed a range of increase in average root diameter from 6.81% (ALB 38, resistant) to 31.87% (ALB89, sensitive) over 48h. We identified four RILs (ALB41, ALB45, ALB87 and ALB43) with fine roots and less inhibition TRER under Al-toxic stress in hydroponic system. However none of them exhibited superior TRL combined with RTP for more extensive root system (Figure 2a), or TRL combined with lower MRD for better exploration of soil profiles (Figure 2b).

The primary site of Al toxicity is the root tip (Ryan et al., 1993; Chen et al., 2006). Working with wheat, Rincon and Gonzales (1992) observed the effects of Al toxicity particularly in root tips and in lateral roots. Root tips represent a significant part of total root surface area which increases contact of roots with soil solution, and are responsible for water and nutrient up-take. In seedlings, it is clear that the root tip is the most sensitive part of the root to Al toxicity (Samac and Tesfaye, 2003). With RILs from DOR364 x G19833 (López-Marín et al., 2009), genotype effects and genotype x treatment interaction were highly significant for TRL and number of root tips. In our study, similar interaction was found with RTP but not TRL. The RTP was strongly reduced by exposure to Al for all genotypes in our experiment.

The relationship between total root length and number of root-tips has been used to identify extensive, well branched root systems. Wagatsuma et al. (1987) reported that Al concentration was high in the roots and generally low in the shoots, and was largely deposited in the root tips in Al sensitive plants. This suggests that root tips are a key site of Al-resistance. However, our results showed that resistant parent G35346-3Q was characterized by low number of root tips and large TRL value. In contrast, the susceptible recurrent parent SER16 showed a favorable combination of both root traits. Best lines in nutrient solution for the combination of these traits were ICA Quimbaya, VAX1, ALB34, ALB46, SER16, ALB88, DOR390, ALB111, ALB58 and ALB13 (Figure 2a). The lines that combined high TRL with low MRD were VAX1, ICA Quimbaya, ALB13, SER16, ALB46, ALB88, ALB121, and DOR390 (Figure 2b). Ironically, the Al sensitive parent SER16 and the sensitive control genotype VAX1 appear in both groups of elite lines, making it difficult to conclude about these traits as criteria to identify Al resistant genotypes.

Total root length and number of root tips per plant were highly correlated ($r = 0.83^{***}$; Table 1) in 20 µM Al. Injuries on root tips have direct effect on whole root system. Mean root diameter was more correlated to number root tips than to TRL, both correlations being negative, respectively $r = -0.65^{***}$ and $r = -0.37^{***}$. As the RTP increased the MRD decreased simultaneously. Liu et al. (2010) concluded that roots of larger (>0.4 mm) diameters only contributed a small proportion to the TRL for the oilseeds. This seems to be an explanation for the high relationship between TRL and RTP (and corresponding small roots) found in our study.

Root growth and acid soil tolerance: Differences in RDP among 102 genotypes have been identified in the soil tube experiment with low and high Al saturation. Effects on RDP in soil cylinders were highly significant for Al-stress, for genotypes, and genotypes x Al treatment interaction (Table 2). Villagarcia et al. (2001) have showed in sand culture that tap root length was not affected greatly by Al treatments. Few researchers have used soil-based method to quantify roots for Al resistance. A modified soil system called soil-on-agar was the most used (Voigt et al., 1997). Under Al stress, RILs averaged 40.8 cm in

rooting depth in soil but showed variable range. Al-induced root-growth inhibition (Rangel et al., 2005; 2007), and RDP could be considered as criteria in screening for Al resistance and acid soil tolerance. The deepest rooting genotype was G35346-3Q penetrating to 64.3 cm deep while sensitive genotype DOR390 in this study reached only 25.2 cm. Genotypic ranking based on rooting depth identified G35346-3Q, ICA Quimbaya, ALB70, ALB65, ALB72, ALB77, ALB91 and ALB103 as the most acid soil tolerant by this criterion. Rooting depth and TRL were highly correlated (r = 0.77***) showing similar stress effects in response to high Al-saturation in the soil. Al not only causes a reduction in the length of the main root, but also changes the entire root architecture (Doncheva et al., 2005). As free Al concentration in acid soils generally increases with depth, Al-resistant cultivars having roots in the more toxic subsoil might be able to obtain soil resources such as water and nutrients from that layer (Bushamuka and Zobel, 1998). Enhanced exploratory capacity of a root system with extensive, deep roots was found in G35346-3Q, ALB91, ALB77, ALB15, ALB88, and ALB40 (Figure3c).

Segregation of resistance among RILs: The genetic dissection of the quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite to allow cost-effective applications of genomics-based approaches to breeding programs aimed at improving the sustainability and stability of yield under adverse conditions (Collins et al., 2008). For average number of root tips, López-Marín et al. (2009) using bean population from the cross DOR364 x G19833 found high percentage of transgression in control and Al treatment. For our study, the population of RILs was created from a single backcross of the F1 of G35346-3Q x SER16 to SER16. Differences among RILs and between RILs and the recurrent parent SER16 reflect introgression from the *P. coccineus* parent. Segregation of most traits suggested quantitative inheritance and often transgressive segregation, implying that both parents possessed complementary genes for some traits. This was not a surprise considering that SER16 was quite good for TRL and RTP in the hydroponic system. Screening with hydroponic system F2 plants from the cross of Young x PI 416937 in soybean, Bianchi et al. 2000 found that progeny exhibited significant ($P < 0.05$) transgressive segregation for tap root extension under 0 µM Al. High transgressive segregation for tap root elongation was also observed in the control solution with a common bean population (López-Marín et al., 2009). In this study, for example, the two parental lines were virtually equal for TRER 24 h in the 20 µM Al at about 1.4 $mm.h^{-1}$ but the progenies ranged from less than 1 $mm.h^{-1}$ to nearly 2 $mm.h^{-1}$ (Figure 1a). Other traits for which the RILs presented transgressive segregation were TRL in 20 µM Al, TRL in 0 µM Al, and TRL in low Al saturation soil. López-Marín et al. (2009) found that percentage of negative transgression was high in both control and Al treatment for TRL while positive transgressive segregation was low. Mapping genes controlling aluminum tolerance in rice, Nguyen et al. (2002) found that the range of progeny means appreciably exceed that of their parents for root length in stress and control and for root length ratio, suggesting transgressive variation among genotypes. In the case of TRL in 20 µM Al in this study, the transgressive segregation was observed with values below the two parents but not above. These observations confirm the complexity of traits for Al resistance or acid soil tolerance, and suggest that recovering the full complement of genes from G35346-3Q would be difficult, especially through a backcross. Furthermore, when plants are subject to Al stress in soil tube screening system, G35346-3Q was the best by far in TRL, and better than any progeny suggesting that inheritance is complex because no line received the full complement of the coccineus genes in backcross and selfing. Crosses among RILs expressing different traits might serve to recombine individual genes from different RILs.

Benefits from evaluation in both hydroponic and soil tube systems: Identifying genotypic differences in Al resistance entailed frequent measurements of TRER between 24h and 72 h (Furlani and Clark, 1981; Horst et al., 1983; Bennet and Breen, 1991). Despite the imposition of stress to approximately the same degree in hydroponics and sand culture, the genotypic variation in Al tolerance was much greater in hydroponics as evidenced by the following: much greater Al x genotype interaction, increased genotypic variation in response to stress, a much wider range in Al tolerance expressed as percentage of control (PC), a lower correlation between genotypic means for Al-free and Al-stress treatments, and a greater correlation between ratings under Al-stress conditions and PC (Villagarcia et al., 2001). In our study, a close relationship ($R^2 = 0.84$) was found between TRL with and without Al treatment in nutrient solution (Figure 3a). This suggests that whatever effect of Al that resulted in much less TRL, was not an effect for which lines expressed resistance. In contrast, in soil tube system, the effects of genotype x Al interaction for TRL were significant, and the relationship between TRL under high and low Al saturation treatment was weak (Figure 3b). TRL seems to discriminates genotypes for their Al resistance in acid soil while in hydroponics it did not.

In their work on root hair of barley, Gahoonia and Nielsen (1997) found agreement between soil and solution culture, and concluded that this could be due to using a nutrient solution with ionic strength similar to that in the soil solution. However, Noble et al. (1982, 1984, 1987) reported in a series of studies that ratings in greenhouse pots did not agree with solution culture results. Large discrepancy between hydroponics-based rating of seedlings and sand-culture-based ratings of plants was also observed when Al tolerance was expressed as PC (Villagarcia et al., 2001). Sartain and Kamprath (1978) and Sapra et al. (1982) compared shoot growth in the greenhouse with root growth in solution culture and found no association between the two. We found similar results in our study. Results from soil tube system and hydroponics system were compared for TRL, correlating results in low Al saturation soil with 0 µM Al nutrient solution, and high Al saturation soil and 20 µM Al solution. No significant correlation was observed between the two methods for controls (r = 0.003 ns), or for Al treatments (r = 0.04 ns) (Figure 4a) suggesting that each technique is distinct and one method cannot be substituted but complimented to the other. ALB88 was very good both for TRL under stress with the two methods. Horst and Klotz (1990) compared 31 soybean genotypes for Al resistance in solution and sand culture and found a low but significant positive correlation (r = 0.43) between genotypic means of the two methods. In our study, a few weak correlations were identified between the two methods: MRD in high Al saturation soil, with TRL (r = -0.29**) and RTP (r = -0.38***) in nutrient solution with 20µM Al. But contrasting results from our previous study on the identification of new sources of resistance to Al stress using both hydroponics and soil tube screening showed that the two methods were highly correlated for TRL in soil tube with high Al saturation and nutrient solution with 20 µM Al (Butare et al., 2010). It appears that the correlation was driven by the presence of accessions of *P. coccineus* which have multiple Al resistance attributes. ALB46, ALB34, ALB88, ALB67, and ALB48 were good in MRD in soil tubes and maintained high TRL and RTP in nutrient solution. Urrea-Gomez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of Al–tolerant cultivars.

One important difference in the two systems used in our study could be the resistance to root elongation that exists in soil but not in hydroponics. Other potential differences could be in the nutritional status of the plants. The ability of roots to adjust their growth and development to environmental factors (Lopez-Bucio et al., 2003; Forde and Lorenzo, 2001) could be an explanation for the differences observed. Seedlings in nutrient solution are evaluated for a short period (for several hours or days) while for soil tube evaluation, plants are grown for several days or even a month or more. P diffuses freely to the root surface in solution culture (Clarkson, 1991), whereas in soil, diffusion of P to the root surface is rate limiting and the zone of soil close to the root is depleted uniformly due to the geometrical arrangement of root hairs on root (Gohoonia and Nielsen, 1991). To successfully select Al-resistant lines that perform well in acid soil, Al-resistance could not be deduced based only on the inhibition of root growth alone but must take into account the effects of Al on the entire plant. Ryan et al. (1994) found that low concentrations of Al could inhibit root growth and Ca uptake. Screening methods and Al concentrations that reveal differences in shoot development could probably improve correlations with performance in acid soil, and selection for Al resistance. The hydroponic system used in this study is suitable for selection for Al resistance alone based only on root development and distribution, while soil tube system revealed differences in root architecture, acquisition of soil resources and pattern of biomass allocation in leaves, steams, and roots.

Root growth requires nutrients that are absorbed from the soil and photosynthates that are transported from the shoot (Lopez-Bucio et al., 2003). A significant Al x genotype interaction indicated genotypic variation in response to the imposition of Al stress for shoot dry weights (Villagarcia et al., 2001). A similar result on shoots was observed in soil tube screening (Table 2) with significant genotype, and genotype x Al level effect ($P < 0.001$). Both TRL and shoot biomass showed genotypic variation in response to the Al-toxic acid soil stress. Rating based on the combination of TRL and shoot dry biomass weight revealed not only the effects of Al-stress in the soil but biomass accumulation in shoot that could translate into yield in the reproductive phase. Interesting lines that maintain a high capacity for biomass accumulation above and below ground in an extensive root system are ALB119, ALB77, G35346-3Q, ALB89, ALB88 and ALB91 (Figure 5a). Among these lines ALB88 was able to produce more leaf area with less biomass which is an advantage in a competitive situation, and it has also high RTP (Figure 5b). The relationship of shoot dry biomass accumulation in soil tubes and a fine root system in hydroponics was evaluated, and although there was no correlation (Figure 5c), ALB77, ALB88, ALB117, ALB58, ALB15, and ALB38 were found to be superior lines that combine high shoot biomass accumulation and fine root (≤0.45mm of MRD) development in nutrient solution.

Many of Al-tolerant plant species release organic acid anions such as citrate, malate or oxalate from root apices in response to Al-stress (Delhaize et al., 1993b; Kidd et al., 2001; Kikui et al., 2005), and organic acid anions that chelate Al cation and ameliorate Al-induced inhibition of root elongation (Ma, 2000; Ma et al., 2001; Kikui et al., 2005). This could be another possible explanation for no expression of resistance in hydroponics if the nutrient solution combined with frequent change of solution diluted the organic acids and erased those differences.

In conclusion, our data reaffirm that the runner bean genotype, G353466-3Q, is an Al resistant material and could be a very good source for improving acid soil tolerance in common bean. Regarding a number of root and shoot traits evaluated using soil tube system; several root parameters expressed well in the best lines for shoot development and may contribute to shoot biomass accumulation of those lines. Introgression of *P. coccineus* to SER16 had changed TRER and total root density of their progeny by improving the resistance to Al-toxic stress. Compared to SER16 also tolerance to Al-toxic acid soil was improved by the introduction of SER16 x G35346-3Q traits. Higher values of TRL and RDP found with ALB91, ALB88, ALB77 and ALB 89 contributed to greater shoot development. The use of both systems could contribute to evaluate breeding materials to identify genotypes that combine Al resistance with acid soil tolerance. However, results on Al resistance obtained from hydroponic system were not correlated well with the data on acid soil tolerance from soil tube system indicating that use of either system alone can eliminate some useful genotypes. Different concentrations of Al in hydroponic system and different levels of Al saturation in soil system could be tested to further test the relationship of root traits with shoot traits. This knowledge will be useful to understand the physiological basis of differences in ranking of genotypes under acid soil field conditions across seasons and years. The results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

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Rferences

Barlow, P.W. 1975. The root cap. *In* Torrey, J.G. and D.T. Clarkson (Eds.) The development and function of roots. Third Cabot Symposium. Ch. 2, pp 21-54. Academic Press, London.

Bennet, R.J., C.M. Breen, and M.V. Fey. 1987. The effects of aluminum on cap function and root development in *Zea mays* L. Environ. Exp. Bot. 27:91-104.

Bennet, R.J. and C.M. Breen. 1989. Towards understanding root growth responses to environment signals: The effect of aluminum on maize. S. Afric. J. Sci. 85:9-12.

Bennet, R.J. and C.M. Breen. 1990. The recovery of the roots of *Zea mays* L. from various aluminum treatments: Towards elucidating the regulatory processes that underlie root growth control. Environ. Exp. Bot. 31(2):153-163.

Bennet, R.J., C.M. Breen, and M.V. Fey. 1991. The aluminum signal: new dimensions of aluminum tolerance. Plant and soil1, 34:153-166.

Bianchi-Hall, C.M., T.E. Carter, Jr., M.A. Bailey, M.A.R. Mian, T.W. Rufty, D.A. Ashley, H.R. Boerma, C. Arellano, R.S. Hussey, and W.A. Parrott. 2000. Aluminum tolerance associated with quantitative trait loci derived from soybean PI 416937 in hydroponics. Crop Sci. 40:538-545.

Blancaflor, E.B., D.L. Jones, and S. Gilroy. 1998. Alterations in the cytoskeleton accompany Aluminum-induced growth inhibition and morphological changes in primary roots of maize. Plant Physiol. 118:159-172.

Bushamuka, V.N., and R.W. Zobel. 1998. Maize and soybean tap, basal, and lateral root responses to a stratified acid, aluminum-toxic soil. Crop Sci. 38:416-421.

Butare, L., I.M. Rao, P. Lepoivre, J. Polania, C. Cajiao and S. Beebe. 2010. New sources of resistance in *Phaseolus* species to individual and combined stress factors of aluminium-toxic acid soil and drought**.** (to be submitted)

Box, J.E. 1996. Modern methods for root investigations. P. 193-237. *In* Y. Waisel et al. (ed.) Plant roots: The hidden half. Marcel Dekker, New York.

Campbell, K.A.G., and T.E. Carter, Jr. 1990. Aluminum tolerance in soybean: I. Genotypic correlation and repeatability of solution culture and greenhouse screening methods. Crop Sci. 30:1049-1054.

CIAT. 2005. Project IP1: Bean Improvement for the tropics. Annual report. 366p. Cali, Colombia.
CIAT. 2007. Bean genomics for improved drought tolerance in Central America. Final report. Cali, Colombia.
Clarkson, D.T. 1965. The effect of aluminium and some other trivalent metal cations on cell division in the root apices of *Allium cepa*. Ann. Botany N. S. 29: 309-315
Clarkson, D.T. 1991. Root architecture and site of ion uptake. *In* Y. Waisel, A. Eshel & U. Kafkafi U (Eds.), Plant roots: The Hidden Half, pp. 417-453. Marcel Dekker, Inc.
Collins, R.P., P.J. Gregory, H.R. Rowse, A. Morgan, and B. Lancashire. 1987. Improved methods of estimating root length using a photocopier, a light box, and a bar code reader. Plant Soil 103:227-280.
Collins, N.C., F. Tardieu, and R. Tuberosa. 2008. Quantitative traits loci and crop performance under abiotic stress: where do we stand? Editor's choice series on the next generation of biotech crops. Plant Physiology 147:469-486.
Cunningham, M., M.B. Adams, R.J. Luxmoore, W.M. Post, and D.L. DeAngelis. 1989. Quick estimates of root length, using a video image analyzer. Can. J. For. Res. 19:335-340.
Delhaize, E., S. Craig, C.D. Beaton, R.J., V.C. Jagadish, and P.J. Randall. 1993a. Aluminum tolerance in wheat (*Triticum aestivum* L.) I. uptake and distribution of Aluminum in roots apices, Plant Physiol. 103:685-693.
Delhaize, E., P.R. Ryan, and P.J. Randall. 1993b. Aluminum tolerance in wheat (Triticum aestivum L.): II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol.103:695-702.
Doncheva, S., M. Amenós, C. Poschenrieder, and J. Barceló. 2005. Root cell patterning: a primary target for aluminum toxicity in maize. Journal of Exp. Bot. 56(414):1213-1220.
Eisenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15:763-782.
Forde B. and Lorenzo H. (2001). The nutritional control of root development. Plant Soil, 232: 51-68.
Foy, C.D., Plant adaptation to acid, aluminium toxic soils, Comm. Soil Sci. Plant Anal. 19 (1988):959-987.
Foy, C.D. 1992. Soil chemical factors limiting plant root growth. p.97-149. *In* Hatfield J.L., Stewart B.A. Adv. (Eds.). Advances in Soil Sciences: Limitations to Plant Root Growth, Vol. 19, Springer Verlag, New York.
Foy, C.D. 1996. Tolerance of Barley cultivars to an acid, aluminium-toxic subsoil related to mineral element concentrations of their shoots, J. Plant Nutr. 19:1361-1380.
Furlani, R.R. and R.B. Clark. 1981. Screening Sorghum for aluminum tolerance in nutrient solution. Agron. J. 73:587-594.
Gahoonia, T.S. and N.E., Nielsen. 1991. A method to study rhizosphere processes in thin soil layers of different proximity to roots. Plant and Soil 135:143-146.
Gahoonia, T.S. and N.E., Nielsen. 1997. Variation in root hairs of barley cultivars doubled soil phosphorus uptake. Euphytica 98:177-182.
Goldman, I.L., Carter, Jr., T.E., and R.P. Patterson, 1989. Differential genotypic response to drought stress and subsoil aluminum in soybean. Crop Sci. 29:330-334.
Hoekenga, O., P. Mason, J. Shaff, E. Buckler, L. Kochian. 2003. Identification and characterization of Al tolerance genes in the intermated B73 x Mo17 population of maize by QTL mapping. Plant Physiol., p. 53 (Abstr.).
Horst, W.J., C.J. Asher, I. Cakmak, P. Szulkiewicz, and A.H. Wissemeier. 1992. Short-term responses of soybean roots to aluminium. J. Plant Physiol. 140:174-178.
Horst, W.J., A.-K. Püschel, and N. Schmohl. 1997. Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. Plant Soil 192:23-30.
Horst, W.J., Y. Wang and D. Eticha. 2010. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Ann. Bot. (in press).
Horst, W.J., and F. Klotz. 1990. Screening soybean for aluminum tolerance and adaptation to acid soils. p. 355-360. *In* N. El Bassan (ed.) Genetic aspects of plant mineral nutrition. Klumer Academic Publisher, Dordrecht, the Netherlands.
Kamprath, E.J. 1984. Crop Responses to lime on soils in the Tropics. p.349-368. *In* Soil Acidity and Liming; Adams, F., Ed.; American Society of Agronomy: Madison, Wisconsin.

Kidd, P.S., M. Llugany, C. Poschenrieder, B. Gunse and J. Barcelo. 2001. The role of root exudates in aluminum resistance and silicon-induced amelioration of aluminum toxicity in three varieties of maize (Zea mays L.). J. Exp. Bot. 52:1339-1352.

Kikui, S., T. Sasaki, M. Maekawa, A. Miyao, H. Hirochika, H. Matsumoto, and Y. Yamamoto. 2005. Physiological and genetic analyses of aluminum tolerance in rice, focusing on root growth during germination. Journal of Inorganic Biochemistry 99:1837-1844.

Kochian, L.V. 1995. Cellular mechanisms of aluminium toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46:237-260.

Kochian, L.V., O.A. Hoekenga, and M.A. Piñeros. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu. Rev. Plant Biol. 2004. 55:459-493

Kochian, L.V., M.A. Piñeros, and O.A. Hoekenga. 2005. The physiology, genetics and molecular biology of plant aluminium resistance and toxicity. Plant and Soil 274:175-195.

Liu L.P., Y. Gan, R. Bueckert, K.Van Rees, and T. Warkentin. 2010. Fine root distribution in oilseed and pulse crops. Crop Sci. 50:222-226.

López-Bucio, J., A. Cruz-Ramírez, and L. Herrera-Estrella. 2003. The role of nutrient availability in regulating root architecture. Current Opinion in Plant Biology, 6:280-287.

López-Marín, H.D., I.M. Rao, and M.W. Blair. 2009. Quantitative trait loci for aluminum toxicity resistance in common bean (*Phaseolus vulgais* L.). Theoretical and Applied Genetics 119: 449-458.

Ma, J.F. 2000. Plant cell Physiol. 41:383-390.

Ma, J.F., P.R. Ryan, and E. Delhaize. 2001. Aluminum tolerance in plants and the complexing role of organic acids. Trends Plant Sci. 6:273-278.

Manrique, G., Rao, I., and S. Beebe. 2006. Identification of aluminium resistant common bean genotypes using a hydroponic screening method. Paper presented at the 18th World Congress of Soil Science, Philadelphia, USA, July 9-15, 2006.

Massot N., M., Llugany, C. Poschenrieder and J. Barcelo, 1999. Callose production as indicator of aluminum toxicity in bean cultivars. J. Plant Nutri. 22, 1-10.

Matsumoto, H. 2000. Cell biology of aluminum toxicity and tolerance in higher plants. International review of cytology, Vol. 200, pp1-46.

Miklas, P.N., J.D. Kelly, S.E. Beebe & M. W. Blair, 2006. Common bean breeding for resistance for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147:105-131.

Narasimhamoorthy, B., E.B. Blancaflor, J.H. Bouton, M.E. Payton, and M.K. Sledge. 2007. A comparison of hydroponics, soil, and root staining methods for evaluation of aluminium tolerance in *Medicago truncatula* (Barel Medic) germplasm. Crop Sci. 47:321-328.

Noble, A.D., M.V. Fey, and J.D. Lea. 1982. Effect of soluble aluminum on seedling root elongation of 25 soybean cultivars. Crop Prod. (Pretoria) 11:119-121.

Noble, A.D., M.V. Fey, and J.D. Lea. 1984. Response of five soybean [*Glycine max* (L.) Merr.] cultivars to lime and phosphorus on an acid Normandian subsoil. S. Afr. J. Plant Soil 1:51-56.

Noble, A.D., M.V. Fey, and J.D. Lea. 1987. Performance of five soybean cultivars in relation to lime and phosphorus levels on an acid ultisol. South Afr. J. Plant Soil 4:140-142.

Pandey, S., H. Ceballos, R. Magnavaca, A.F.C. Bahia Filho, J. Duquevargas, and L.E. Vinasco.1994. Genetics of tolerance to soil acidity in tropical maize. *Crop Sci*. 34:1511-1514.

Rangel, A. F., I. M. Rao and W. J. Horst. 2009. Cellular distribution and binding state of aluminum in root apices of common bean (*Phaseolus vulgaris* L.) genotypes differing in aluminum resistance. Physiologia Plantarum 135:162-173.

Rangel A.F., Rao I.M., and W.J**.** Horst. 2007. Spatial aluminium sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes with contrasting aluminium resistance. J. Exp. Bot. 58:3895-3904.

Rangel A. F., Mobin M., Rao I.M., and W.J. Horst. 2005. Proton toxicity interferes with the screening of common bean (*Phaseolus vulgaris* L.) genotypes for aluminium resistance in nutrient solution. J. Plant Nutr. Soil Sci. 168:607-616.

Rao, I.M., R.S. Zeigler, R. Vera and S. Sarkarung. 1993. Selection and breeding for acid-soil tolerance in crops: Upland rice and tropical forages as case studies. BioScience 43: 454-465.

Raper, C.D.J., D.L. Osmond, M. Wann, and W.W. Weeks. 1978. Interdependence of root and shoot activities in determining nitrogen uptake rate of roots. Bot. Gaz. (Chicago) 139:289-294.

Rincon, M., and R.A. Gonzales. 1992. Aluminum partitioning in intact roots of aluminum-tolerant and aluminum-sensitive wheat (*Triticum aestivum* L.) cultivars. Plant Physiol. 99:1021-1028.
Rout G.R., S. Samantaray and P. Das. 2001. Aluminium toxicity in plants: a review. INRA, EDP Science. *Agronomie* 21 (2001) 3-21.
Ryan, P. and E. Delhaize. 2010. The convergent evolution of aluminium resistance in plants exploits a convenient currency. Funct. Plant Biol. 37: 275-284.
Ryan P.R., J.M. DiTomaso, and L.V. Kochian. 1993. Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44:437-446.
Ryan P.R., T.B. Kinraide, and L.V. Kochian. 1994. Al^{3+}-Ca^{2+} interactions in aluminum rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. Planta 192:98-103.
Samac, D.A., and M. Tesfaye. 2003. Plant improvement for tolerance to aluminum in acid soils-A review. Plant Cell Tissue Organ Cult. 75:189-207.
Sapra, V.T., T. Mebrahtu, and L.M. Mugwira. 1982. Soybean germplasm and cultivar aluminum tolerance in nutrient solution and Blanden clay loan soil. Agron. J.74:687-690.
Sartain, J.B., and E.J. Kamprath. 1978. Aluminum tolerance of soybean cultivars based on root elongation in solution culture compared with growth in acid soil. Agron J 70:17-20.
Shen, H., X. Yan, K. Cai, and H. Matsumoto. 2004. Differential Al resistance and citrate secretion in the tap and basal roots of common bean seedlings. Physiologia Plantarum 121:595-603.
Sivaguru, M., and W.J. Horst. 1998. The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. Plant Physiology 116:155-163.
Stass, A., Z. Kotur, and W. J. Horst. 2007. Effect of boron on the expression of aluminum toxicity in *Phaseolus vulgaris*. Physiol. Plant 131: 283-290.
Stutte, G.W., and E.C. Stryjewski. 1995. Computer classification of roots from digitized video images. HortScience 30:906 (abstr.).
Urrea-Gómez, R., H. Ceballos, S. Pandey, A.F.C Bahía Filho, and L.A. León. 1996. A greenhouse screening technique for acid soil tolerance in maize. Agron. J. 88:806-812.
Villagarcia, M.R., T.E. Carter, Jr., T.W. Rufty, A.S. Niewoehner, M.W. Jennette, and C. Arrellano. 2001. Genotypic ranking for aluminum tolerance of soybean roots grown in hydroponics and sand culture. Crop Sci.41:1499-1507.
Voigt, P.W., D.R. Morris, and H.W. Godwin. 1997. A soil-on-agar method to evaluate acid soil resistance of while clover. Crop Sci. 37:1493-1496.
Wagatsuma, T., T. Kyuunda, and A. Saturaba. 1987. Aluminium accumulation characteristics of alumnium-tolerant plants, Bull. Yamagata Univ. Agric. Sci. 10:355-359.
Wenzl, P., L.I. Mancilla, J.E. Mayer, R. Albert and I. M. Rao. 2003. Simulating infertile acid soils with nutrient solutions and the effects on *Brachiaria* species. Soil Sci. Soc. Am. J. 67: 1457-1469.

4. Phenotypic evaluation of drought resistance in recombinant inbred lines (RILs) of DOR 364 x BAT 477 under intermittent drought stress

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Rationale: We evaluated drought adaptation of 33 RILs of the cross DOR 364 x BAT 477 over two seasons (2005 and 2006) under terminal drought stress. In 2007, we evaluated 97 RILs of the cross DOR 364 x BAT 477 under intermittent drought stress to obtain phenotypic data for eventual gene tagging for drought resistance.

Materials and Methods: A field trial was conducted at Palmira in 2007 (June to September). The soil is a Mollisol (Aquic Hapludoll) with no major fertility problems (pH = 7.7), and is estimated to permit storage of 130 mm of available water (assuming 1.0 m of effective root growth with –0.03 MPa and –1.5 MPa upper and lower limits for soil matric potential). The trial included 97 RILs of DOR 364 x BAT 477 along with 1 check (SEA 5) and 2 parents (DOR 364, BAT 477) to determine genotypic differences in tolerance to drought stress conditions. A 10 x 10 balanced lattice design with 3 replicates was used. Two levels of water supply (irrigated and rainfed) were applied. For the irrigated treatment, a total of 4 gravity irrigations (approximately 35 mm each) were applied while for the rainfed treatment only 2 irrigations were applied to assure good crop establishment. Experimental units consisted of 2 rows, 3.72 m long by 0.6 m wide. A number of plant attributes were measured at mid-podfilling under both rainfed and irrigated conditions in order to determine genotypic variation in drought resistance. These plant traits included leaf chlorophyll content (SPAD), canopy temperature, canopy temperature depression (CTD), leaf area index, canopy dry weight per plant and shoot TNC content (total nonstructural carbohydrates).

Canopy temperature was measured with a Telatemp model AG-42D infrared thermometer. The instrument was held at a 45^{o} angle at 50 cm from the canopy surface to measure canopy temperature and canopy temperature depression. At the time of harvest, grain yield and yield components (number of pods per plant, number of seeds per pod, and 100 seed weight) were determined. Stem biomass reduction (mobilization of photosynthate reserves) was determined based on difference in stem dry weight at harvest from the stem dry weight at mid-pod filling. Pod partitioning index (dry wt of pods at harvest/dry wt of total biomass at mid-podfill x 100), pod harvest index (dry wt of seed/dry wt of pod at harvest x 100), pod number per area, seed number per area, yield production efficiency (seed biomass dry weight at harvest/total shoot biomass dry weight at mid-pod filling), seed production efficiency (seed number per area/ total shoot biomass dry weight at mid-pod filling per area), pod production efficiency (pod number per area/ total shoot biomass dry weight at mid-pod filling per area) and grain filling index (100 seed weight of rainfed/100 seed weight of irrigated) were determined. Shoot and seed TNC (total nonstructural carbohydrate) contents were also measured.

Results and Discussion: During the crop-growing season, maximum and minimum air temperatures were 30.56 and 18.61 ^{o}C (Figure 1). The incident solar radiation ranged from 11.2 to 25.1 MJ m^{-2} d^{-1}. The total rainfall during the active crop growth was 243.1 mm (a significant portion falling during grainfilling). The potential pan evaporation was of 431 mm. These data on rainfall and pan evaporation together with rainfall distribution indicated that the crop suffered intermittent drought stress during active growth and development. The mean yield under rainfed conditions was 849 kg ha^{-1} compared with the mean irrigated yield of 1741 kg ha^{-1} showing 51% decrease in mean grain yield due to drought stress (Figure 2).

Under drought stress conditions in the field, the seed yield of 97 RILs ranged from 603 to 1171 kg ha^{-1} (Figure 2). Among the RILs tested, two RILs BT 21138-68-1-1 and BT 21138-74-1-1 were outstanding in their adaptation to rainfed (water stress) conditions. The relationship between grain yield of rainfed and irrigated treatments indicated that several RILs were superior to the best parent, BAT 477 and the check genotype, SEA 5. Among the 97 lines tested, BT 21138-31-1-1 and BT 21138-34-1-1 were found to be very poorly adapted lines under rainfed conditions.

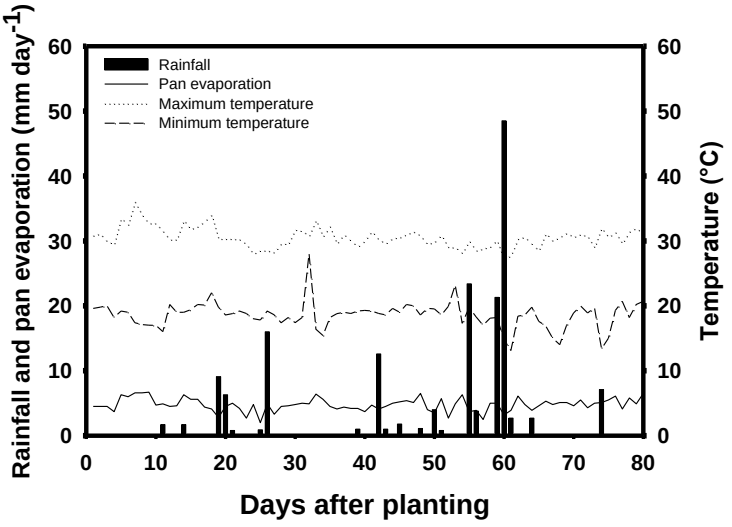

Figure 1. Rainfall distribution, pan evaporation, maximum and minimum temperatures during crop growing period at Palmira in 2007.

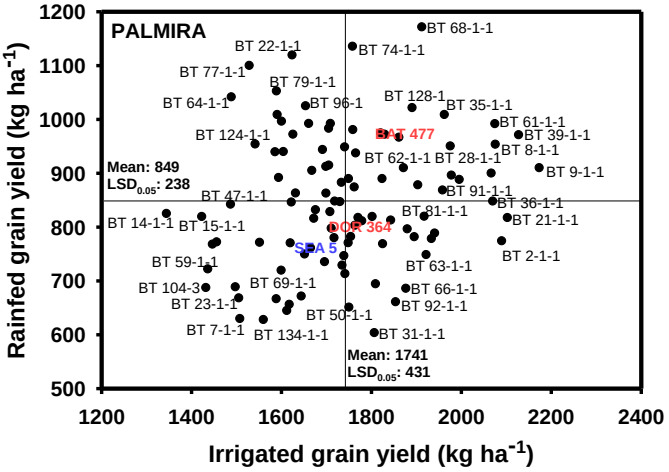

Figure 2. Identification of genotypes that are adapted to rainfed conditions and are responsive to irrigation in a Mollisol at Palmira. Genotypes that yield superior with drought and were also responsive to irrigation were identified in the upper, right hand quadrant.

Significant genotypic differences were observed in canopy biomass production at mid-pod filling growth stage (Figure 3). The RILs BT 21138-64-1-1 and BT 21138-69-1-1 showed greater vigor than the rest of the RILs; however these RILs showed lower value of harvest index indicating a limitation on mobilization of photosynthates to pod and seed development. The relationship between rainfed seed yield and other plant attributes showed that the outstanding performance of lines BT 21138-68-1-1 and BT 21138-74-1-1 was associated with higher values of stem biomass reduction indicating greater mobilization of photosynthates from stems to pods (Figure 4).

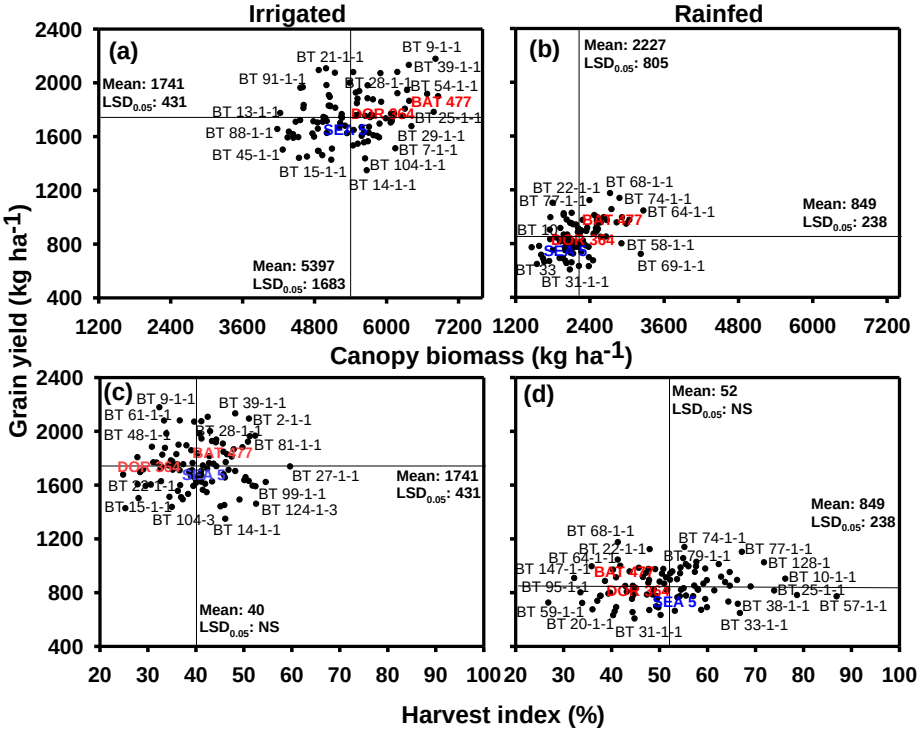

Figure 3. The relationship between grain yield and irrigated and rainfed canopy biomass (a, b) and grain yield and irrigated and rainfed harvest index (c, d) when grown in a Mollisol at Palmira.

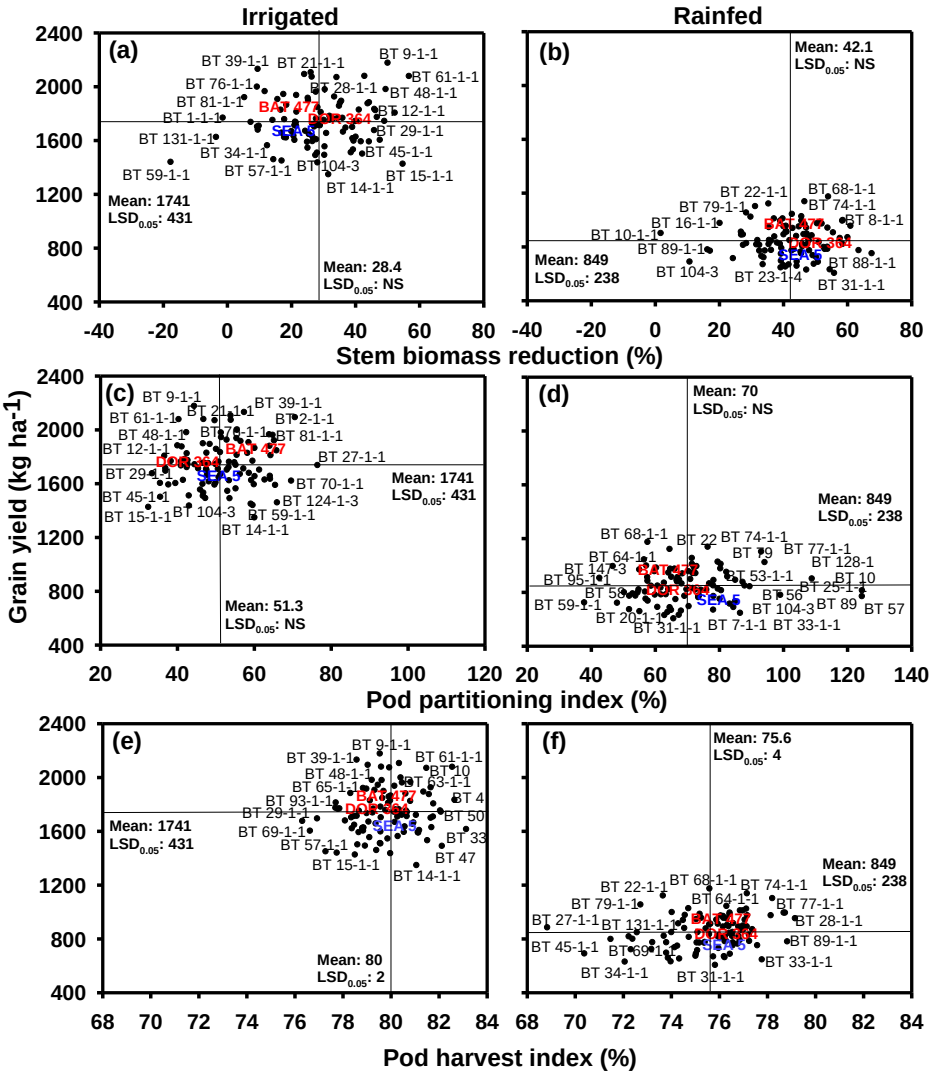

Figure 4. The relationship between grain yield and irrigated and rainfed stem biomass reduction (a, b), grain yield and irrigated and rainfed pod partitioning index (c, d), and grain yield and irrigated and rainfed pod partitioning index (e, f) when grown in a Mollisol at Palmira.

The relationship between rainfed seed yield and seed number per area, pod number per area and 100 seed weight showed that the line BT 21138-74-1-1 was outstanding in producing greater number of seeds and pods and also in filling the grain (Figure 5).

Correlation coefficients between final grain yield and other shoot attributes under rainfed conditions indicated significant relationship between seed yield and leaf area index and canopy biomass under irrigated and rainfed conditions (Table 1). It is important to note that the harvest index, pod partitioning index, pod harvest index, seed number per area and pod number per area showed significant positive association with seed yield under rainfed conditions. This indicates that the genotypes that mobilized a greater proportion of photosyntates from plant structures to pod and seed formation performed better under rainfed conditions (Figures 4 and 5).

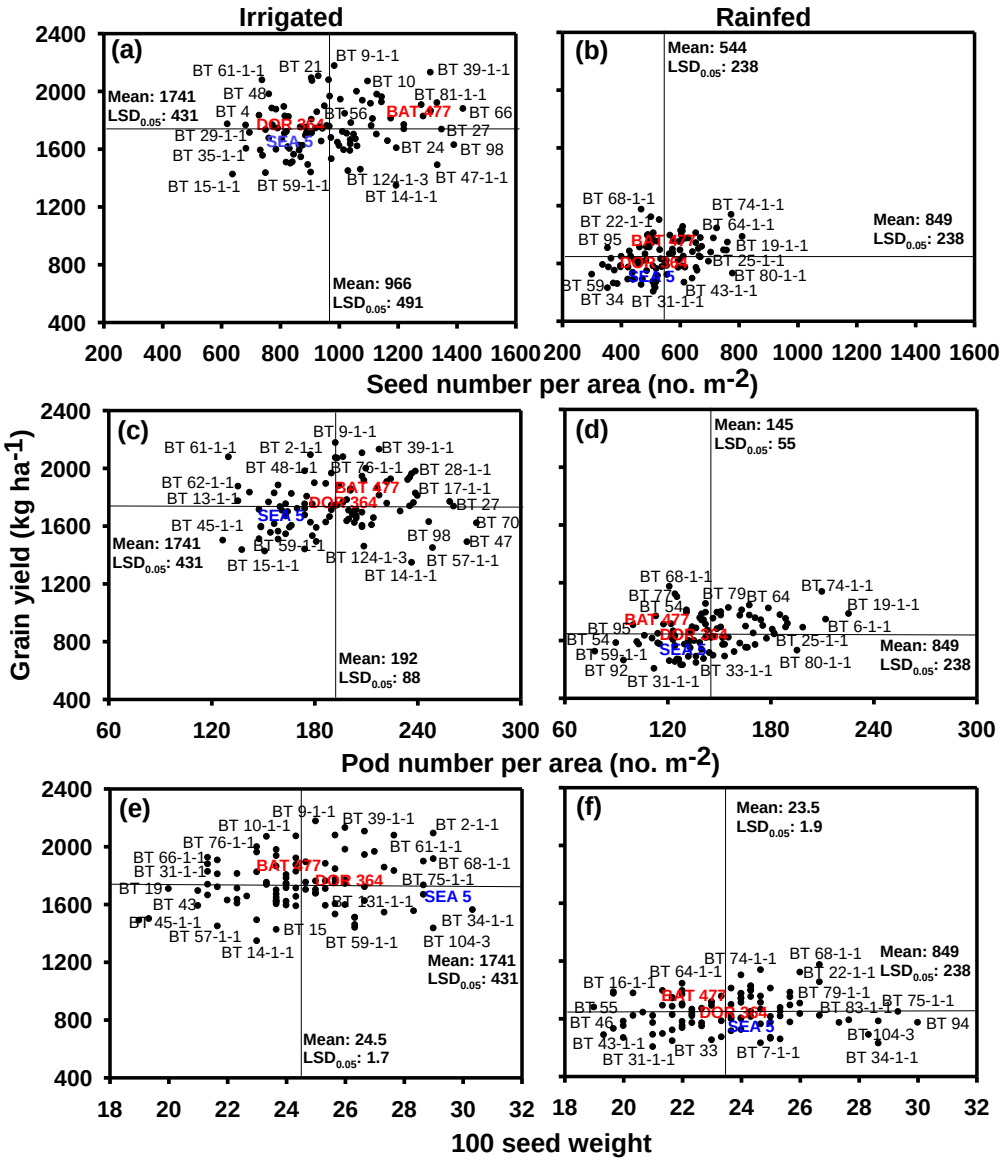

Figure 5. The relationship between grain yield and irrigated and rainfed seed number per area (a, b),grain yield and irrigated and rainfed pod number per area (c, d), and grain yield and irrigated and rainfed 100 seed weight (e, f) when grown in a Mollisol at Palmira.

Table 1. Correlation coefficients (r) between final grain yield (kg/ha) and other plant attributes of RILs of common bean grown under irrigated and rainfed conditions in a Mollisol in Palmira.

Plant traits	Irrigated	Rainfed
Leaf area index (m^2/m^2)	0.19***	0.34**
Total chlorophyll content (SPAD)	-0.12*	0.12*
Canopy biomass (kg ha^{-1})	0.38***	0.33***
Canopy temperature (^oC)	-0.13*	-0.08
Canopy temperature depression (^oC)	0.16**	0.09
Shoot TNC content (mg g^{-1})	0.10	-0.02
Seed TNC content (mg g^{-1})	-0.09	-0.02
Pod partitioning index (%)	0.02	0.11*
Harvest index (%)	0.03	0.13*
Pod harvest index (%)	0.11*	0.19***
Stem biomass reduction (%)	0.04	-0.10
Seed number per area (No m^2)	0.23***	0.35***
Pod number per area (No m^2)	0.12*	0.31***
Seed number per pod	0.17**	0.13*
Days to flowering	0.07	-0.05
Days to maturity	0.14*	0.08
100 seed weight (g)	0.11*	0.11*
Grain filling index (%)		0.12*

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Conclusions: Field evaluation of 97 RILs of the cross DOR 364 x BAT 477 under intermittent drought stress resulted in identification of two RILs BT 21138-68-1-1 and BT 21138-74-1-1 that were outstanding in adaptation to intermittent drought stress conditions. The superior performance of these lines under intermittent drought stress was associated with higher values of harvest index, pod partitioning index, stem biomass reduction, seed number per area and pod number per area indicating the importance of greater mobilization of photosyntates to pods and seeds under rainfed conditions.

5. Phenotypic evaluation for deep rooting ability and drought resistance in recombinant inbred lines (RILs) of DOR 364 x BAT 477

Contributors: J. Polania, M. Grajales, C. Cajiao, R. Garcia, J. Ricaurte, S. Beebe and I. Rao

Rationale: We evaluated 97 RILs of the cross DOR 364 x BAT 477 under terminal drought stress to obtain phenotypic data on deep rooting ability for eventual gene tagging for drought resistance.

Materials and methods: A greenhouse study was conducted at CIAT - Palmira using a mix of an Andisol (from Darien of Colombia) with river sand (2:1 w/w). Plants were grown for 48 days in plastic cylinders (80 cm long with 7.5 cm diameter) inserted in PVC tubes from October to December 2007. Soil cylinders were carefully packed with 4200 g of soil: sand mixture, with bulk density of 1.2 g cm^{-3}. The trial included 97 RILs of the cross DOR 364 x BAT 477 and two parents and one check SEA 5 to determinate genotypic differences in root development and distribution under drought stress. The trial was planted as a randomized complete block arrangement with two levels of water supply: 80% field capacity (well-watered) and withholding of watering (to simulate terminal drought stress conditions) as main plots and genotypes as sub-plots with three replications. Soil was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). Treatments of water stress were imposed after 10 days of initial growth of plants that were established with seed previously germinated in paper. The initial soil moisture for all the treatments was of 80% field capacity. Plants with well-watered treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder. Plants with terminal drought were monitored for water stress by weighing each cylinder every two days for determination of decrease in soil moisture. Plants were harvested at the age of 48 days after establishment, i.e., 38 days of withholding of water application.

A number of physiological characteristics were measured during the experiment. This included total chlorophyll content (SPAD), leaf temperature and rooting depth. At the time of harvest (48 days after establishment; 38 days of water stress treatment), leaf area; shoot biomass distribution, and root traits were determined. The soil from the tube was removed and sliced into 6 layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75). Roots in each soil layer were washed free of soil and sand and root length, mean root diameter, specific root length, and root dry weight (gm) were determined. Root length and mean root diameter were measured with an image analysis system (WinRHIZO, Regent Instruments INC, v.2003 b). Root weight was determined after roots were dried in an oven at 60^{o}C for 48 h. Analysis of variance was calculated by using the SAS computer program (SAS/STAT, 2001). A probability level of 0.05 was considered statistically significant.

Results: Soil moisture and temperature: During the crop-growing season, the average of maximum and minimum air temperatures were 39.68^{o}C and 20.53^{o}C (Figure 1); with a maximum photon flux density of 1100 µmol m^{-2} s^{-1}; Soil bulk density of 1.2 g cm^{-3}. The final soil moisture for the plants under terminal drought was 28% of field capacity.

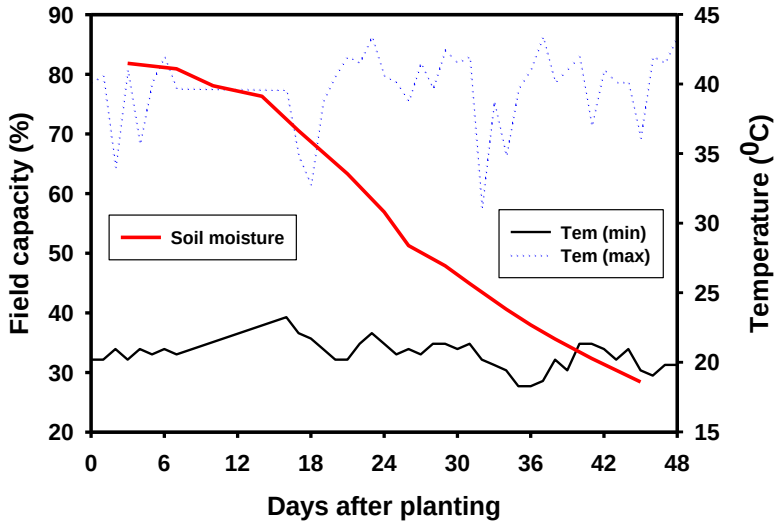

Figure 1. Soil moisture (field capacity), maximum and minimum temperatures during soil drying and root development in the soil tube in greenhouse at Palmira in 2007.

No significant genotypic differences were observed in terms of shoot biomass production in both control and terminal drought conditions. Water stress conditions markedly decreased the biomass of leaves, stem and pods of 100 genotypes tested when compared with well-watered treatment (Figure 2). Shoot biomass production under terminal drought conditions was relatively less affected for the lines BT 3-1-1, BT 16-1-1 and BT 29-1-1. Among the 100 genotypes tested under terminal drought stress, BT 124-1-1, BT 41-1-1 and BT 9-1-1 were the poorest shoot biomass production.

Results on total root length showed significant genotypic differences under both irrigated and terminal drought conditions (Figure 3). The genotypes BT 34-1-1, BT 79-1-1 and BT 77-1-1 were outstanding in their total root length under terminal drought. Among the 100 genotypes tested BT 69-1-1, BT 6-1-1 and BT 74-1-1 had poor root development under terminal drought conditions. Under irrigated conditions BT 10-1-1, BT 20-1-1 and BT 21-1-1 showed the highest total root length and BT 81-1-1, DOR 364 and BT 35-1-4 were the lowest total root length under irrigated conditions (Figure 3). Results on specific root length showed significant genotypic differences under both irrigated and terminal drought stress (Figure 4). The genotypes BT 9-1-1, BT 124-1-1 and BT 80-1-1 were outstanding in thin roots developing under terminal drought stress conditions, while the genotypes BT 36-1-1, BT 72-1-1 and BT 85-1-1 had thicker roots under terminal drought stress.

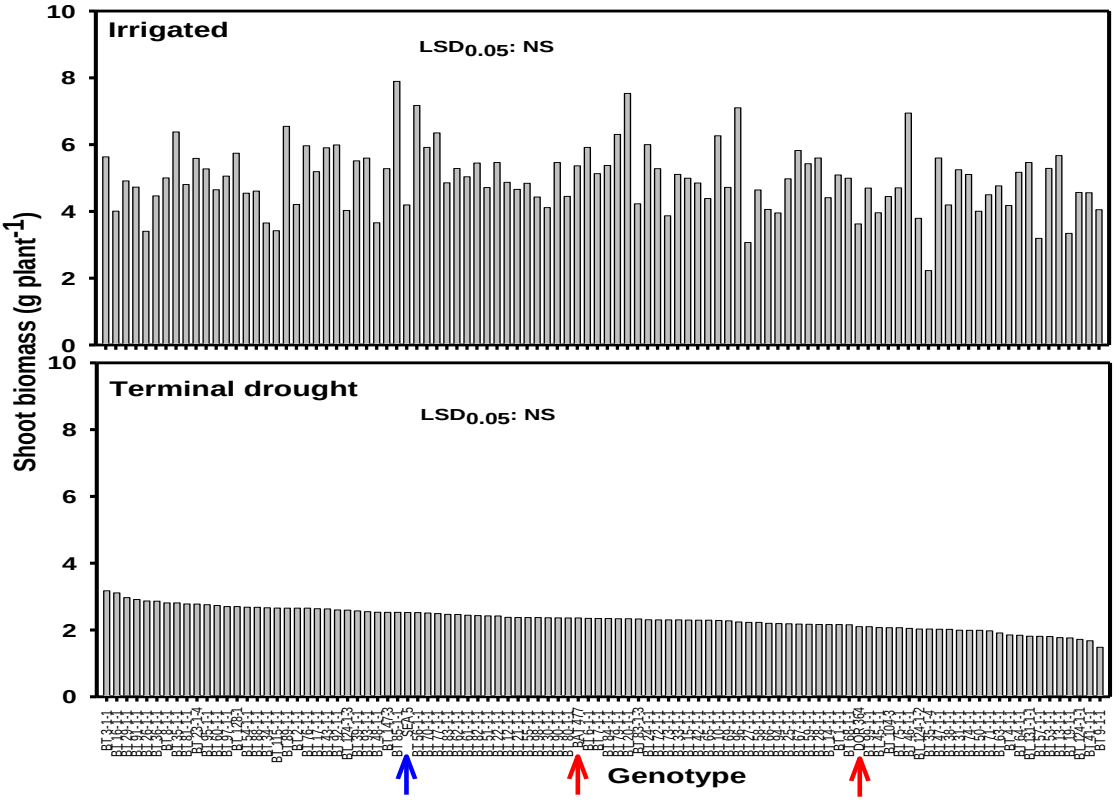

Figure 2. Influence of irrigation and terminal drought stress on shoot biomass of 100 genotypes of the cross DOR 364 X BAT 477 under greenhouse conditions, Palmira in 2007

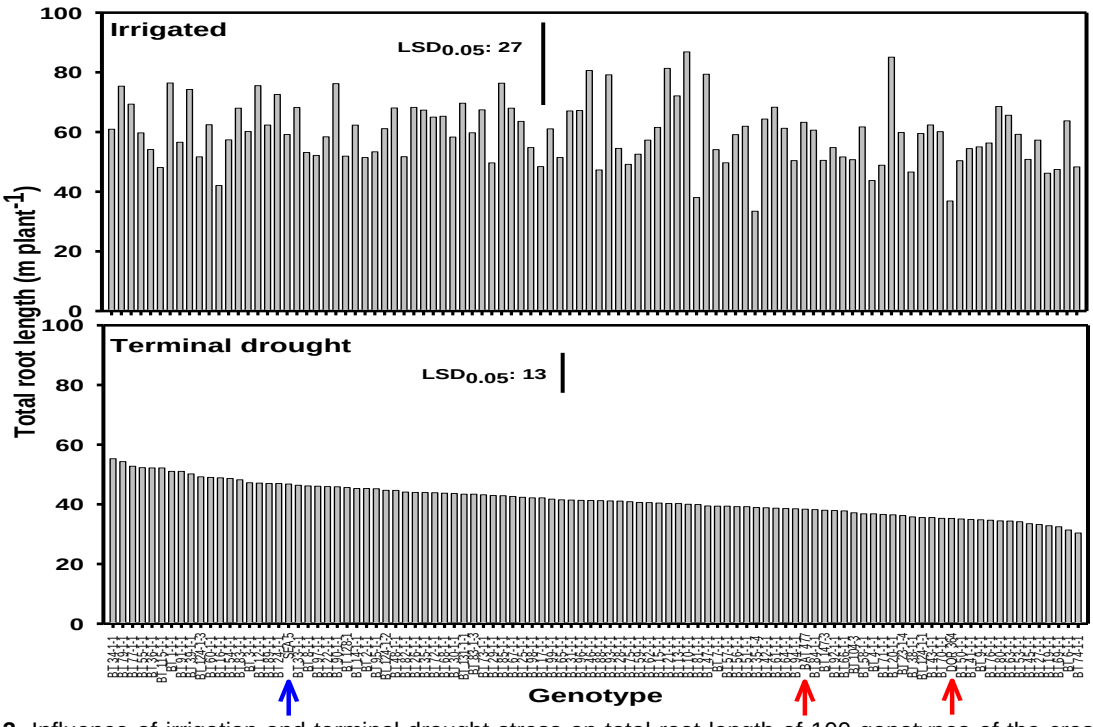

Figure 3. Influence of irrigation and terminal drought stress on total root length of 100 genotypes of the cross DOR 364 X BAT 477 under greenhouse conditions, Palmira in 2007

Significant genotypic differences were observed in terms of depth rooting under both irrigated and water stress conditions. The genotypes BT 34-1-1, BT 68-1-1, SEA 5 and BT 83-1-1 were outstanding in their depth rooting ability at 35 days after planting under drought conditions. The genotypes BT 53-1-1, BT 9-1-1 and BT 50-1-1 showed the lowest depth rooting under terminal drought stress.

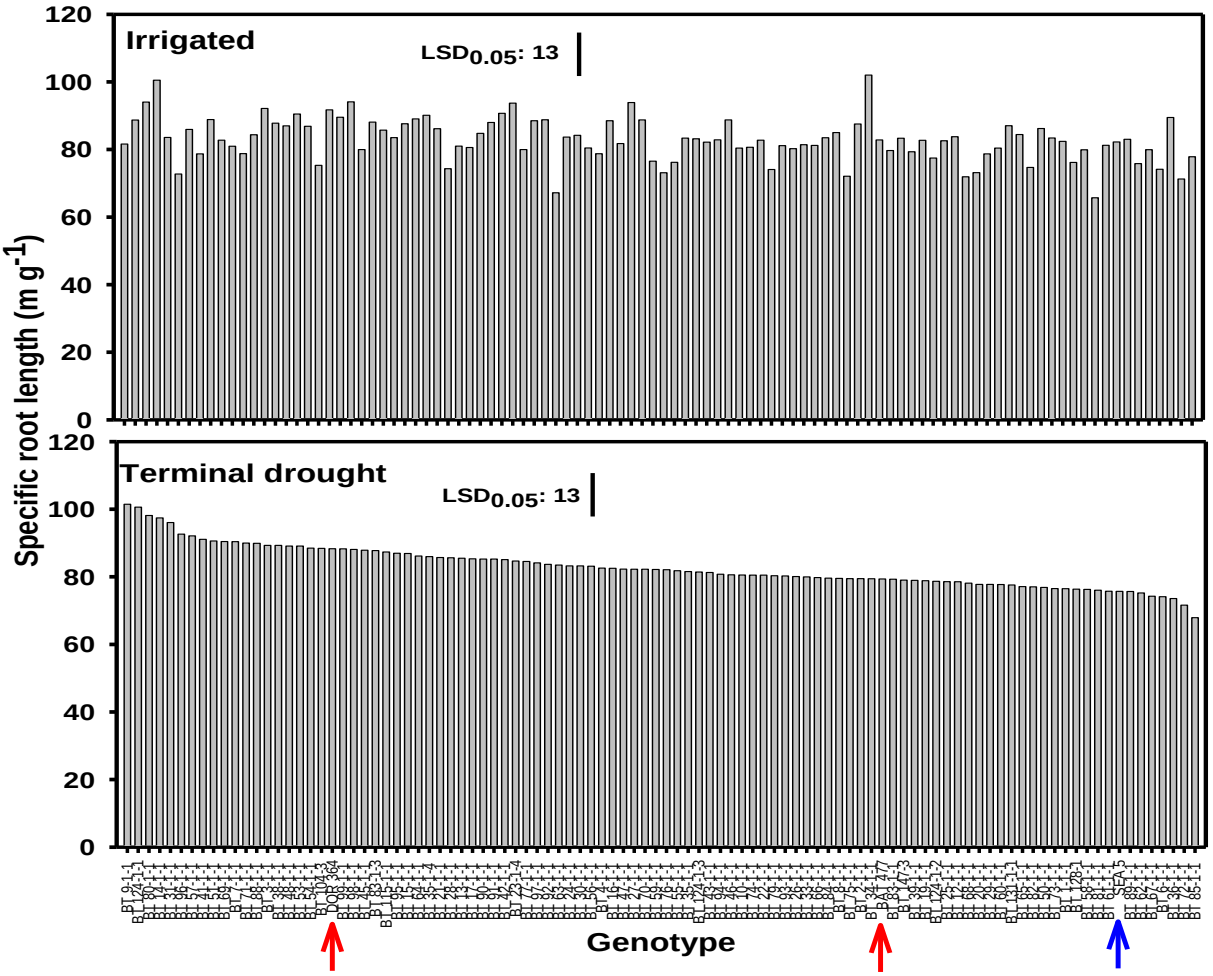

Figure 4. Influence of irrigation and terminal drought stress on specific root length of 100 genotypes of the cross DOR 364 X BAT 477 under greenhouse conditions, Palmira in 2007

The genotypes BT 34-1-1, BT 91-1-1 and BT 85-1-1 were outstanding in their root development at soil depth 60-75 cm under terminal drought stress. The genotypes BT 80-1-1, BT 104-3, BT 35-1-4 and BT 51-1-1 were the lowest root length at soil depth 60-75 cm under terminal drought stress (Figure 5). Under irrigated conditions the genotypes BT 131-1-1, BT 1-1-1 and BT 10-1-1 were outstanding in their root length production at soil depth 60-75 cm.

Correlation coefficients between grain yield in the field over 3 seasons and root traits from the greenhouse study indicated that greater grain yield under rainfed conditions was positively related to total root length, mean root diameter, root volume, deep rooting at 35 days after planting and root length at soil depth of 60-75 cm (Table 1). This observation indicates that several superior performers under drought developed deeper roots and greater amount of fine roots.

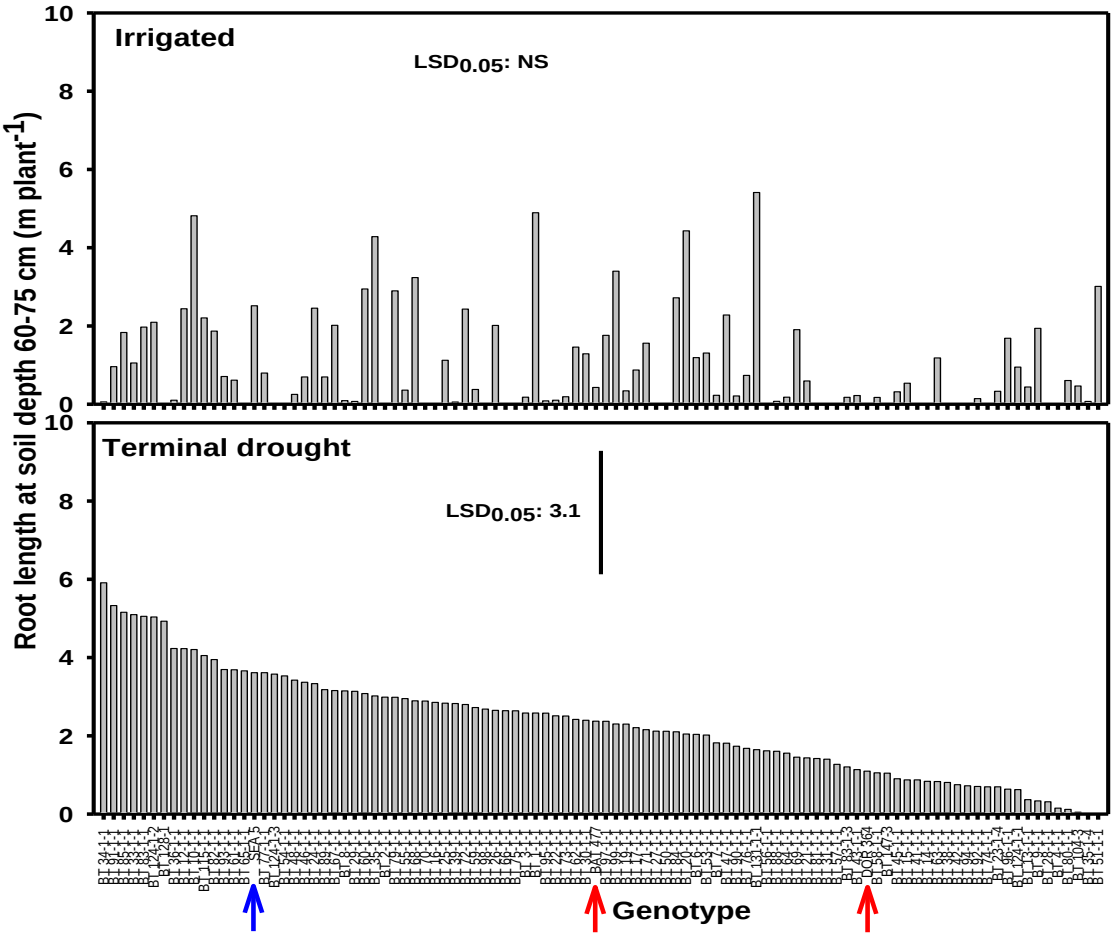

Figure 5. Influence of irrigation and terminal drought stress on root length at soil depth 60-75 cm of 100 genotypes of the cross DOR 364 X BAT 477 under greenhouse conditions, Palmira in 2007

Table 1. Correlation coefficients (r) between final grain yield (kg/ha) in three seasons and root attributes 100 genotypes of the cross DOR 364 X BAT 477 under greenhouse conditions, Palmira in 2007.

Root traits	Irrigated	Rainfed
Total root length (m plant^{-1})	0.04	0.12*
Mean root diameter (mm)	0.12*	0.14*
Root volume (cm^3)	0.07	0.17**
Specific root length (m g^{-1})	-0.15**	-0.07
Deep rooting at 35 days after planting (cm)	0.11	0.17**
Root length at soil depth 60-75 cm	0.01	0.14*

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Conclusions: Greenhouse evaluation of 100 bean genotypes using soil tube method for root phenotyping resulted in identification of four genotypes BT 34-1-1, BT 79-1-1, BT 77-1-1 and BT 55-1-1 that were superior in their total root development under terminal drought stress conditions. The genotypes SEA 5, BT 83-1-1, BT 91-1-1, BT 29-1-1 and BT 61-1-1 were superior in their development of deep roots. The superior performers under drought stress developed a more vigorous root system and deeper roots. It appears that greater rooting depth and access to moisture alone will not assure good yield under drought. Further work is needed under field conditions to verify this observation.

ANNEX 5

Project Title

Fighting drought and aluminum toxicity: *Integrating functional genomics, phenotypic screening and participatory evaluation with women and small-scale farmers to develop stress-resistant common bean and Brachiaria for the tropics*

Project Supported by
Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)

Executed by
International Center for Tropical Agriculture (CIAT)
in collaboration with
University of Hannover, Germany
Institut des Sciences Agronomiques du Rwanda (ISAR), Rwanda
National Department of the Ministry of Agriculture (DARS), Malawi
Instituto Nicaragüense de Tecnología Agropecuaria (INTA), Nicaragua

Reporting Period
April 2006 – March 2010

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ANNEX 5

List of Publications, Papers, Reports and Theses

ANNEX-5

List of Publications, Papers, Reports and Theses

The project staff members have published a total of 21 journal articles, 2 book chapters, 6 articles in conference proceedings and 37 oral and poster presentations at international and national conferences and workshops. A total of 10 students (2 PhD, 3 MSc, 5 BSc) theses were approved by different universities in Germany, Rwanda, Malawi and Colombia.

Refereed journal articles

Butare, L., I. M. Rao, P. Lepoivre, C. Cajiao, J. Polania, J. B. Cuasquer and S. Beebe. 2010. Phenotypic evaluation of interspecific recombinant inbred lines (RILs) of *Phaseolus* species for their resistance to aluminum and tolerance to aluminum-toxic acid soil under greenhouse conditions. Euphytica (to be submitted).

Butare, L., I. M. Rao, P. Lepoivre, J. Polania, C. Cajiao, J. B. Cuasquer and S. Beebe. 2010. New sources of resistance in *Phaseolus* species to individual and combined stress factors of aluminum-toxic soil and drought. Crop Sci. (in review).

Blair, M. W., C. H. galeano, E. Tovar, M. C. Muñoz Torres, A. Velasco, S. Beebe and I. M. Rao. 2010. Development of a Mesoamerican intra-genepool genetic map for QTL detection in a drought tolerant x susceptible common bean (*Phaseolus vulgaris* L.) cross. Theoretical and Applied Genetics (in review).

Horst, WJ., Wang, Y., Eticha, D. 2010. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Annals of Botany 106: 185-197.

Yang, Z., D. Eticha, I. M. Rao and W. J. Horst. 2010. Alteration of cell-wall porosity is involved in osmotic stress-induced enhancement of aluminium resistance in common bean (*Phaseolus vulgaris* L.). J. Exp. Bot. (in press). doi:10.1093/jxb/erq146 (JXB Advance Access published online).

Eticha, D., M. Zahn, M. Bremer, Z. Yang, I. M. Rao and Walter Horst. 2010. Transcriptomic analysis reveals differential gene expression in response to aluminium in common bean (*Phaseolus vulgaris* L.) genotypes. Ann. Bot. 105: 1119-1128.

Rao, I. M., P. Wenzl, A. Arango, J. Miles, T. Watanabe, T. Shinano, M. Osaki, T. Wagatsuma, G. Manrique, S. Beebe, J. Tohme, M. Ishitani, A. Rangel and W. Horst. 2008. Advances in developing screening methods and improving aluminum resistance in common bean and *Brachiaria*. Revista Brasileira de Agrociencia 14 (4) (in press).

Rangel, A. F., I. M. Rao, H. P. Braun and W. J. Horst. 2010. Aluminum resistance in common bean (*Phaseolus vulgaris* L.) involves induction and maintenance of citrate exudation from root apices. Physiol. Plant. 138: 176-190.

Blair M.W. and M. Ishitani. 2009. Common bean: a model food legume for international agriculture. Grain Legumes 53: 8-9

López-Marín, H.D., M.W. Blair and I. M. Rao. 2009. Identification of aluminum resistant Andean genotypes of common bean (*Phaseolus vulgais* L.). Braz. J. Plant Physiol. 21 (4): 291-300.

López-Marín, H.D., I.M. Rao and M.W. Blair. 2009. Quantitative trait loci for aluminum toxicity resistance in common bean (*Phaseolus vulgais* L.). Theoretical and Applied Genetics 119: 449-458.

Polanía, J., I. M. Rao, S. Beebe and R. García. 2009. Desarrollo y distribución de raices bajo estrés por sequía en frijol común (*Phaseolus vulgaris* L.) en un sistema de tubos con suelo. Agronomia Colombiana 27: 25-32.

Rangel, A. F., I. M. Rao and W. J. Horst. 2009. Cellular distribution and binding state of aluminum in root apices of common bean (*Phaseolus vulgaris* L.) genotypes differing in aluminum resistance. Physiologia Plantarum 135: 162-173.

Beebe, S., I. M. Rao, C. Cajiao and M. Grajales. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Science 48: 582-592.

Rangel, I. M. Rao and W. Horst. 2007. Spatial aluminum sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes that are contrasting in aluminium resistance. J. Exp. Bot. 58(14):3895-3904.

Ricaurte, J., I. M. Rao and C. Menjívar. 2007. Estrategias de enraizamiento de genotipos *Brachiaria* en suelos ácidos y de baja fertilidad en Colombia. Acta Agronómica (Colombia) 56(3):107-115.

Begum, H. H., M. Osaki, M. Nanamori, T. Watanabe, T. Shinano and I. M. Rao. 2006. Role of phosphoenolpyruvate carboxylase in the adaptation of a tropical forage grass, *Brachiaria* hybrid, to low phosphorus acid soils. J. Plant Nutrition 29: 35-57.

Wenzl, P., A. Arango, A. L. Chaves, M. E. Buitrago, G. M. Patiño, J. Miles and I. M. Rao. 2006. A greenhouse method to screen brachiariagrass genotypes for aluminum resistance and root vigor. Crop Sci. 46: 968-973.

Watanabe, T., M. Osaki, H. Yano and I. M. Rao. 2006. Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages. J. Plant Nutrition 29: 1243-1255.

Rao, I. M., J. W. Miles, R. García y J. Ricaurte. 2006. Selección de hibridos de *Brachiaria* con resistencia a aluminio. Pasturas Tropicales 28: 20-25.

Hausler, K., I. M. Rao and R. Schultze-Kraft and late H. Marschner. 2006. Shoot and root growth of two tropical grasses, *Brachiaria ruziziensis* and *B. dictyoneura* as influenced by aluminum toxicity and phosphorus deficiency in a sandy loam Oxisol of the eastern plains of Colombia. Trop. Grasslands 40: 213-221.

Book chapters

Beebe, S., J. Ramirez, A. Jarvis, I. M. Rao, G. Mosquera, J. M. Bueno and M. Blair. 2010. Genetic improvement of common beans and the challenges of climate change. In: S. S. Yadav, B. Redden, J. L. Hartfield and H. Lotze-Campen (eds.) Crop Adaptation to Climate Change. John Wiley & Sons, Inc. (in review).

Beebe, S. E., I. M. Rao, M. W. Blair and J. A. Acosta-Gallegos. 2009. Phenotyping common beans for adaptation to drought. Generation Challenge Program Special Issue on Phenotyping, pp. 311-334.

Conference proceedings

Beebe, S., I.M. Rao, C. Cajiao, M.A. Grajales, and L. Butare. 2010. Yield potential from interspecific crosses of common and runner bean. Ann. Rept. of the Bean Improv. Coop. 53:26-27.

Eticha, D, Marc Zahn, Idupulapati M. Rao, and Walter J. Horst (2009): Transcriptomic analysis reveals differential gene expression in common bean (*Phaseolus vulgaris*) for aluminum resistance. The Proceedings of the International Plant Nutrition Colloquium XVI. Paper1150. http://repositories.cdlib.org/ipnc/xvi/1150.

Beebe, S., I. Rao, M. Blair and L. Butare. 2009. Breeding for abiotic stress tolerance in common bean: present and future challenges. Proceedings of the 14th Australian Plant Breeding & 11th SABRAO Conference, 10 to 14 August, 2009, Brisbane, Australia (invited paper).

Horst, W. J., A. F. Rangel, D. Eticha, M. Ishitani and I. M. Rao. 2009. Aluminum toxicity and resistance in *Phaseolus vulgaris* – physiology drives molecular biology. Proceedings of the

7th International Symposium on Plant-Soil Interactions at Low pH, May 17-21, 2009, Guangzhou, China (invited paper).

Beebe, S., I. M. Rao, J. Polania, M. Grajales and C. Cajiao. 2008. Improved harvest index in drought resistant common beans and possible effects on combining ability. Bean Improvement Cooperative. Annual Report, pp. 8-9.

Beebe, S., I. M. Rao, H. Terán and C. Cajiao. 2006. Breeding concepts and approaches in food legumes: The example of common bean. In: Food and forage legumes of Ethiopia: Progress and prospects. Proceedings of the workshop on food and forage legumes. Addis Ababa, Ethiopia. 22-26 September, 2003. ICARDA, Aleppo, Syria, pp. 23-29.

Oral and poster presentations

Rao, I. M., J. Rincón, J. Polania, J. Ricaurte, V. H. Villegas, R. García, G. Borrero, J. A. Cardoso and J. Miles. 2010. Evaluación y selección de gramíneas tropicales con adaptación a suelos ácidos, sequía y exceso de agua. Paper presented at I National course on resistance to biotic and abiotic stress factors organized by the Colombian Association of Breeding and crop production. 2-4 June, 2010. CIAT, Cali, Colombia (Invited).

Rao, I. M., S. E. Beebe, J. Polania, M. Grajales, C. Cajiao, J. Ricaurte, G. Borrero and M. Rivera. 2010. Avances en caracterización fenotípica en adaptación a sequía en frijol común. Paper presented at I National course on resistance to biotic and abiotic stress factors organized by the Colombian Association of Breeding and crop production. 2-4 June, 2010. CIAT, Cali, Colombia (Invited).

Beebe, S., I. Rao, M. Blair and L. Butare. 2009. Mejoramiento para tolerancia al Estrés Abiótico en Frijol Común: desafios actuales y futuros. Paper presented at I National course on resistance to biotic and abiotic stress factors organized by the Colombian Association of Breeding and crop production. 2-4 June, 2010. CIAT, Cali, Colombia (Invited).

Rao, I. M., S. E. Beebe, J. Polania, M. Grajales, C. Cajiao, J. Ricaurte, G. Borrero and M. Rivera. 2010. Phenotypic evaluation of crop adaptation to abiotic stress factors in the tropics: Common bean as a case study. Paper presented at the Consultants Meeting on "Development of Mutant Germplasm for Enhancing Crop Productivity with better Adaptation to Climate Change"held from 10-14 May, 2010 at IAEA, Vienna, Austria (Invited).

Ricaurte, J., J. Rincón, J. Polania, V. H. Villegas, G. Borrero, J. A. Cardoso, J. Miles and I. M. Rao. 2010. Evaluación y selección de gramíneas tropicales con adaptación a suelos ácidos, sequía y exceso de agua. Paper presented at IAEA Training Course on Methods of Evaluation of Mutants. February 1-5, 2010, CIAT, Cali, Colombia.

Polania, J., M. Grajales, C. Cajiao, J. Ricaurte, M. Rivera, M. Blair, S. E. Beebe and I. M. Rao. 2010. Avances en caracterización fenotípica en adaptación a sequía en frijol común. Paper presented at IAEA Training Course on Methods of Evaluation of Mutants. February 1-5, 2010, CIAT, Cali, Colombia.

Eticha D, Rangel AF, Heinze H, Zahn M, Rao IM, and Horst WJ (2009) Transcriptomic analysis reveals differential gene expression in common bean for aluminum resistance. A poster presented on the 16th International Plant Nutrition Colloquium (IPNC), Sacramento CA, USA. 26 – 30 Aug. 2009.

Eticha D, Braun H-P, Rao IM, and Horst WJ (2009) Proteomic changes in root tips of Aluminium stressed common bean (*Phaseolus vulgaris* L.). A poster presented on Annual Meeting of the German Plant Nutrition Society (DGP), Osnabrück, Germany. 11 – 12 June 2009.

Yang, ZB, D Eticha, IM Rao, WJ Horst (2009) Osmotic stress improves aluminium-resistance in *Phaseolus Vulgaris* L. by changing cell wall porosity. Abstract and Poster in Annual Conference of the German Society of Plant Nutrition. pp71

Rane, J., I. Rao, M. Ishitani and J. Tohme. 2009. Phenotyping platform at CIAT: Improving abiotic stress tolerance of crops and forages in the tropics. Paper presented at Interdrought

III: The 3rd International Conference on Integrated Approaches to Improve Crop Production Under Drought-Prone Environments, October 11-16, 2009, Shanghai, China.

Rao, I. M., S. E. Beebe, J. Polanía, M. Grajales, C. Cajiao, R. García, J. Ricaurte and M. Rivera. 2009. Physiological basis of improved drought resistance in common bean: the contribution of photosynthate mobilization to grain. Paper presented at Interdrought III: The 3rd International Conference on Integrated Approaches to Improve Crop Production Under Drought-Prone Environments, October 11-16, 2009, Shanghai, China.

Beebe, S., I. Rao, M. Blair and L. Butare. 2009. Mejoramiento para tolerancia al Estrés Abiótico en Frijol Común: desafios actuales y futuros. Invited paper presented at the 2do Congreso Internacional de Frijol. Zacatecas, Mexico. August, 2009 (Invited).

Rincón, J., J. Polania, V. Hoyos, J. Ricaurte, R, García, J. Miles and I. M. Rao. 2009. Evaluación y selección de gramíneas tropicales con adaptación a sequía y exceso de agua. Paper presented at the International Seminar on Climate Change and Livestock Systems. March 24-25, 2009, CORPOICA, Bogota, Colombia.

Mutimura, M. and Everson, T.M. 2009. Assessment of livestock feed resource-use patterns in low rainfall and aluminium toxicity prone area of Rwanda. In: Grassland Society of Southern Africa (GSSA), 44th Annual Congress: Meeting Rangeland, Pasture and Wildlife Challenges in a Changing Landscape. 20-25th July 2009, University of South Africa, Roodepoort, South Africa. Book of Abstracts, pp 82–83.

Polanía, J. A., M. Grajales, C. Cajiao, R. García, J. Ricaurte, S. Beebe and I. M. Rao. 2008. Physiological evaluation of drought resistance in elite lines of common bean (*Phaseolus vulgaris* L.) under field conditions. Poster paper presented at Knowledge Sharing Week at CIAT. Poster was awarded second place.

Ricaurte, J., I. M. Rao y J. C. Menjivar. 2008. Enraizamiento de cultivares e híbridos de *Brachiaria* en épocas lluviosas y secas y su impacto en calidad del suelo en los Llanos Orientales de Colombia. Paper presented at the XIV Colombian Congreso of Soil Science at Villavicencio, Meta, Colombia. 28 to 31 October, 2008. (poster presentation).

Yang, ZB, D Eticha, IM Rao, WJ Horst (2008) The interaction between aluminium toxicity and drought stress in common bean (*Phaseolus vulgaris* L.). Abstract and Poster in Annual Conference of the German Society of Plant Nutrition. 23-24 September 2008, Page 39

Eticha D., I. M. Rao and W. J. Horst. 2007. Interaction between aluminium toxicity and drought stress in common bean (P*haseolus vulgaris* L.) genotypes. Poster presented on the Annual Meeting of the German Society of Plant Nutrition – DGP in Berlin, Germany.

Rao, I. M., S. Beebe, J. Ricaurte, C. Cajiao, J. Polania and R. Garcia. 2007. Phenotypic evaluation of drought resistance in advanced lines of common bean (*Phaseolus vulgaris* L.). Paper presented at ASA-CSSA-SSSA International Annual Meeting, New Orleans, LA, USA. 4-8 November, 2007.

Rangel, A. F., I. M. Rao and W. J. Horst. 2007. Spatial aluminum sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes with contrasting aluminum resistance. In: Jahrestagung der Deutschen Gesellschaft für Pflanzenernährung, Berlin, Humbolt-Universität zu Berlin, 57.

Rao, I. M., J. Polania, R. García and S. Beebe. 2007. Desarrollo de un método en invernadero usando tubos con suelo para cuantificar diferencias fenotípicas en desarrollo y distribución de raíces en líneas avanzadas de fríjol común bajo condiciones de estrés por sequía. Paper presented at the LIII Reunión Anual de PCCMCA (Program Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales), Antigua Guatemala, Guatemala. 23-27 April, 2007.

Polania, J., I. M. Rao, S. Beebe and R. García. 2007. Evaluación del desarrollo y distribución de raíces bajo estrés por sequía en 16 genotipos de frijol común (*Phaseolus vulgaris* L.) usando cilindros plasticos en condiciones de invernadero. Paper presented at the XXXVII Congreso

Anual de COMALFI (Sociedad Colombiana de Control de Malezas y Fisiologia Vegetal), Santa Marta, Colombia. 2-4 May, 2007.

Rincón, J., J. A. Polania, I. M. Rao, J. Miles and R. García. 2007. Variación genotipica por tolerancia a sequía en *Brachiaria* bajo condiciones de invernadero usando un sistema de cilindros plásticos transparentes. Paper presented at the XXXVII Congreso Anual de COMALFI (Sociedad Colombiana de Control de Malezas y Fisiologia Vegetal), Santa Marta, Colombia. 2-4 May, 2007.

Rao, I.M. 2006. Best bet root traits of common bean for impact in a breeding program. Presentation in the Bean Breeding Strategy Workshop. CIAT, Cali, CO, 22-25 August 2006.

Rao, I.M. 2006. Progress in physiology of drought resistance in common bean. Presentation in the Bean Breeding Strategy Workshop. CIAT, Cali, CO, 22-25 August, 2006.

Rao, I.M., S. Beebe, J. Ricaurte, G. Manrique, J. Polania and R. García. 2006. Progress in identifying shoot and root traits of common bean that contribute to improved adaptation to abiotic stress factors. [presentation] [CD-ROM]. In: Bean Workshop on Cultivation of Common Bean in Low Input Systems: Bridging Expectations, Accomplishments and Further Needs (BEAN) (2006, Cali, Colombia). 2006. Presentations [CD-ROM] . Centro Internacional de Agricultura Tropical (CIAT), Cali, CO.

Rao, I. M., S. Beebe, J. Miles, P. Wenzl, A. Rangel and W. Horst. 2006. Advances in physiological aspects of abiotic stress adaptation in Beans and *Brachiaria*. Presentation in the Kick-off workshop of the BMZ-GTZ funded drought and aluminum toxicity project. Kigali, Rwanda, 26-28 June, 2006.

Rao, I. M. 2006. Overview of the BMZ-GTZ Project. Fighting drought and aluminum toxicity: Integrating functional genomics, phenotypic screening and participatory research with women and small-scale farmers to develop stress-resistant common bean and *Brachiaria* for the tropics. Presentation in the Kick-off workshop of the BMZ-GTZ funded drought and aluminum toxicity project. Kigali, Rwanda, 26-28 June, 2006.

Blair, M.W., L.M. Rodriguez, L. Galindo, M. Ishitani, S.E. Beebe, I.M. Rao. 2006. Characterization of DREB genes as drought tolerance candidates in Common Beans (*Phaseolus vulgaris* L.). Annual General Meeting of Generation Challenge Program, Sao Paulo, Brazil, 12-16 September, 2006.

Rangel, A.F., I.M. Rao, W.J. Horst. 2006. Short and long term root-growth responses to aluminium in common bean (Phaseolus vulgaris L.). Poster presented at “Plant Nutrition meets Plant Breeding”, first joint conference of the German Society of Plant Nutrition – DGP and the Research Centre for Biotechnology and Plant Breeding Uni. Hohenheim – FSP, Hohenheim University, Stuttgart, Germany.

Rao, I. M., S. Beebe, J. Polania, J. Ricaurte, C. Cajiao y R. García. 2006. Evaluación de resistencia a sequía en lineas recombinante (RILs) de la cruza MD 23-24 X SEA 5. Paper presented at the LII Reunión Anual de PCCMCA (Program Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales), Montelimar, Nicaragua. 24-28 April, 2006.

Beebe, S., I. M. Rao, M. Blair, E. Tovar, M. A. Grajales y C. Cajiao. 2006. Identificación de QTL para resistencia a sequía en lineas recombinante (RILs) de la cruza MD 23-24 X SEA 5. Paper presented at the LII Reunión Anual de PCCMCA (Program Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales), Montelimar, Nicaragua. 24-28 April, 2006.

Beebe, S., I. M. Rao, M. A. Grajales y C. Cajiao. 2006. Evaluación de lineas desarrolladas para resistencia a sequía en condiciones de bajo fósforo en Darién, Colombia. Paper presented at the LII Reunión Anual de PCCMCA (Program Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales), Montelimar, Nicaragua. 24-28 April, 2006.

Rao, I. M., S. Beebe, J. Ricaurte, H. Teran, C. Cajiao, G. Manrique, J. Polania, Y. L. Lopez y M. Blair. 2006. Limitaciones edaficas y climaticas para la producción de frijol comun (*Phaseolus*

vulgaris L.). Paper presented at the Seminario de Bioteconologia y ciencias agrarias. Universidad Nacional, Medellin, Colombia. 31 October-I November, 2006.
Rao, I. M., J. Miles, P. Wenzl, J. Ricaurte, C. Plazas and R. Garcia. 2006. Avances en el desarollo de hibridos de *Brachiaria* con adaptación a suelos ácidos. Paper presented at the Seminario de Bioteconologia y Ciencias Agrarias. Universidad Nacional, Medellin, Colombia. 31 October-I November, 2006.
Manrique, G., I. M. Rao and S. Beebe. 2006. Identification of aluminum resistant common bean genotypes using a hydroponic screening method. Paper presented at the 18th World Congress of Soil Science, Philadelphia, USA. July 9-15, 2006 (Oral and poster paper).
Wenzl, P., A. Chaves, M. Buitrago, G. Patino, J. Miles and I. M. Rao. 2006. Development and validation of a hydroponic screening method to identify acid soil adapted genotypes of the tropical forage grass *Brachiaria*. Paper presented at the 18th World Congress of Soil Science, Philadelphia, USA. July 9-15, 2006 (Oral and poster paper).

PhD, MSc, Diploma and BSc theses

Andres F. Rangel. 2007. Short and medium term effects of aluminum toxicity and resistance in common bean (*Phaseolus vulgaris* L.). PhD thesis. Leibniz University Hannover, Germany.
Louis Butare. 2010. Intraspecific improvement of common bean for higher productivity on soils presenting biotic and abiotic stresses. PhD thesis. University of Liege, Gembloux Agro-Biotech, Belgium.
Martin Emilio Rodriguez Mosquera. 2008. Aisalamiento y caracterización de secuencias de genes expresadas diferencialmente en Brachiaria decumbens Staff cv. Basilisk, asociadas a la resistencia al estrés por aluminio (Al^{3+}) en suelos ácidos. MSc thesis. National University of Colombia, Colombia.
Mupenzi Mutimura. 2009. Evaluation of improved *Brachiaria* grasses in low rainfall and aluminium toxicity prone areas of Rwanda. MSc thesis. University of Kwazulu-Natal, Pietermaritzburg, South Africa, 179 pp.
Bello Shano. 2009. Field Evaluation of common bean lines bred for tolerance to aluminum toxicity and drought. MSc thesis. Bunda College, University of Malawi, Lilongwe, Malawi, 80 pp.
Caroline Quignon. 2007. Hohe Aluminium Toleranz in Brachiaria Spezies. BSc thesis. Leibniz University Hannover, Germany
Nadine Heinze. 2009. Physiologische und molekulare Charakterisierung genotypischer Unterschiede in der Aluminium-Resistenz von *Zea mays* und *Phaseolus vulgaris*. BSc thesis. Leibniz University Hannover, Germany
Valerio Hoyos Villegas. 2007. Physiological screening of Brachiaria spp. Genotypes for their tolerance to drought. BSc thesis. University of Caldas, Caldas, Colombia.
Domitille Mukankubana. 2007. Farmers organization analysis in Gasharu and Sogwe watersheds. BSc thesis. Catholic Institute of Kabgayi, Kigali, Rwanda.
Twahirwa Emmanuel. 2009. Adaptability of Climbing and Bush bean Varieties in Semi-Arid Nyagatare District. BSc thesis. Kigali, Rwanda.